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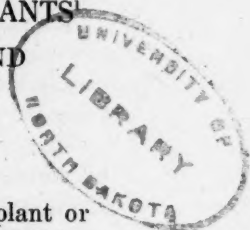
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GROWTH AND DIFFERENTIATION IN PLANTS¹ REGENERATION, DEVELOPMENT AND GENOTYPE

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THE observable characters of a many-celled plant or animal are expressions of the inherent potentialities which together constitute its genotype. The potentialities were transmitted to the individual through the single cell from which its development can be traced. This first cell—spore, zygote or body cell of the parent—will be referred to as the “originating cell,” avoiding the hypothetical connotations that have attached to the term “germ cell.” It follows that the genotype of a many-celled plant is the genotype of its originating cell.

The latter statement is wholly true, of course, only if in the course of the plant’s development its genotype has not been modified. Certain environmental influences can induce changes in chromosome number, changes in chromosome constitution or gene mutations, and hence modifications of the genotype of the affected cells. The commonest visible type of such change consists in a doubling (which may be repeated) of the chromosome number in some or many cells. Tetraploidy, resulting from a doubling of the typical diploid number, is a common occurrence, at least in roots. Ordinarily the “spontaneous” appearance of tetraploid cells must be assumed to

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result from an unrecognized stimulus. It might be argued, but rather quibblingly, that such a response is itself an expression of inherent genotypic possibilities.

However, in some species, including hemp, melons and a number of *Chenopodiaceae*, tetraploidy (disomy) and octoploidy (tetrasomy) are regularly characteristic of certain tissues (Ervin, 1941). Here the change seems pretty clearly not to result from external stimuli. It is in a real sense itself an expression of the plant's genotype.

To what extent does a doubling of chromosome number as distinguished from other chromosomal or genic changes constitute a genotypic modification? This question becomes important when it is considered that from a cell or group of cells with the changed chromosome number a whole tetraploid or octoploid plant may be adventively developed. As to the nature of the genotype of such a plant as compared with that of its diploid progenitor, resort must for the present be made to polyploids produced by deliberately applied means. From these it appears that the distinguishing characters of a tetraploid offspring are essentially quantitative—changes, usually increases, in size of cells and of their parts, with resultant changes in size of organs and of the plant as a whole. But such quantitative modifications sometimes lead to changes in appearance which are easily interpretable as qualitative. For example, many of the cells of *Oenothera gigas* are proportionally more greatly increased in length than in breadth as compared with those of the parent *O. Lamarckiana*. In consequence the forms of organs, including leaves, are modified; the leaves are more crinkly than those of the parent form (Gates, 1915). Straub (1940) finds that similar quantitative modifications due to polyploidy lead in *Antirrhinum*, *Torenia* and *Impatiens* to conspicuous changes in shape and orientation of floral parts and in the distribution of floral pigments; and in *Lupinus* to a marked pubescence of leaves and stems. We are reminded that it has been contended in

some of the older theoretical discussions that all qualitative evolutionary changes are basically quantitative.

In what follows, all effects of mutation, even though of limited extent, are to be excluded; solely to be considered is development based upon the original genotypic capital.

Only very infrequently do two individuals possess precisely similar genotypes. But strains exist whose members are genetically so similar that for practical purposes they may be considered together. Plant clones are illustrations. The genotype of such a strain is naturally thought of first as expressed by the "normal" character of its members—the complex of qualities displayed under usual conditions. But these qualities are consequences of the interaction between genotype and environment; and the character of another member of the strain subjected to another set of conditions may be very different. The "normal" character of an organism then is but a partial expression of the range of possibilities represented by its genotype. How partial that expression may be, only extended experiment can determine. It was a failure—natural enough fifty or sixty years ago—to comprehend this fact that led to the tiresome and meaningless controversies over the inheritance or non-inheritance of "acquired characters."

In order to know the full extent of a plant's genotype it would be necessary to expose that plant, or individuals genetically identical, to every non-lethal combination of conditions. While the complete test remains only theoretically desirable, much of the information suggested by it is available from two sources.

First, with respect to particular genes or gene-combinations, genetic experiment shows that conspicuous differences in phenotype occur under diverse conditions. Genes which make chlorophyll-production possible express themselves only in the presence of light. Similarly, a sun-red coloration in maize appears only in light and is lost through weathering (Emerson, 1921).

Second is the knowledge of genotypic possibilities supplied by physiological experiment. In such work, it is

true, care has not usually been taken to insure that the members of a species subjected to varied conditions are thoroughly comparable genotypically. Hence the results are to be accepted with qualifications. But, with all possible allowances for such uncertainties, the general nature of the results is convincing.

Differences in all the conspicuous characters of a plant follow upon differences in such environmental conditions as the available supply of essential nutrient elements or the form in which these are furnished; the supply of elements whose influence is stimulatory rather than nutritive; or of vitamins and auxins. Other effective external influences are differences in temperature, in atmospheric constitution including humidity, in the physical and chemical make-up of the soil, in intensity, wavelength and time of incidence of illumination.

Interesting light upon genotypic possibilities is thrown when particular stimuli induce the occurrence of growths classed as nodules, intumescences or galls. The remarkable form and structure of some insect galls especially manifest potentialities inherent in the plant but not expressed under usual conditions.

Evidently, then, the genotype of any cell which can give rise to a plant includes a very wide range of developmental possibilities; the plant's "normal" character, so-called, expresses only a small fraction of its inherent capacities; and, apart from mutation, any deviation from this character is in no real sense abnormal; it is at most unusual.

More difficult is the question of the genotype of each of the tissue ("somatic") cells which constitute a plant—all of course derived through division from the originating cell. Reference to this problem recalls the conception most clearly though not originally formulated by Weismann (1883) and still surprisingly influential, of a sharp distinction between germ and somatic cells. The hypothetical basis for this distinction was the supposed occurrence during ontogeny of a series of qualitative nuclear divisions (Weismann, 1885). The postulated conse-

quences of the progressive simplification of the cellular genotype were two-fold. First, a germ cell, capable in itself and its descendants of indefinite division, is "potentially immortal"; a somatic cell, whose power of division and that of its descendants are limited, is necessarily "mortal." Second, a germ cell can by division give rise to all the varied cells and tissues of a complete individual; the potentialities of a somatic cell are limited to the production of cells more or less like itself.

The notion of a series of qualitative nuclear divisions resulting in a progressive simplification of the nuclear content of somatic cells has of course long been untenable. Despite the regular occurrence of chromatin-diminution at certain stages in certain animals, and despite possible mitotic disturbances in any organism by external influences, an outstanding fact in both plant and animal ontogeny is the regular transmission to each cell of a chromosomal complement exactly like that which characterized the originating cell. If the genotype of any cell of a later generation is less comprehensive than that of the originating cell, the explanation is not to be found in a modified nuclear constitution.

Of the two classes of evidence supposed to demonstrate a genotypic difference between germ and somatic cells, the first, that of the necessary mortality of somatic cells, is now in large measure discredited even as to animals by the results of the culture *in vitro* of excised tissues. It is shown that the cells of many or most of the essential tissues of the metazoan body are capable under appropriate conditions of indefinite division. In some instances (Harrison, 1928) division is preceded by dedifferentiation.

The second supposition, of the lack in somatic cells of the totipotence characteristic of germ cells, has been weakened by the accumulation of experimental results summarized under the very broad term "regeneration." Certain of these results were known to Weismann and led to some caution in his statements, particularly regarding plants. More were known to De Vries when he wrote his

"Intracellular Pangenesis" (De Vries, 1889), and many more are now available.

The problem of the genotype of any of the cells of varied structure which constitute the plant body is similar to that of the genotype of the originating cell. The answer is conceivably to be supplied by an examination of the variety of cells, tissues and organs which, under the most varied conditions, can arise from the cell in question in consequence of repeated divisions. But since any approximation to an answer depends upon the ability of the particular cell to divide, it is necessary first to consider how widespread among plant cells is this power of division.

In the filamentous algae and fungi the problem hardly exists, for any cell of one of these plants, at least while young, can divide. Essentially the same is true of the gametophytes of the simpler hepatics, such as *Sphaerocarpos* or *Riccardia*. In more massive plants with differentiated tissues, the condition is less simple. Here each cell begins its life as an element of a meristem. The meristems of a plant—distinct regions of root or stem apex, the cambium, and so on—are somewhat different one from another. While, therefore, it is not true that meristematic cells are wholly undifferentiated, nevertheless such differentiations as they may have undergone, apart from those of size and shape, are relatively slight. What characterizes all meristems is the possibility of repeated cell division.

Some meristems, like those of stem and root apices, persist throughout the life of the plant, or at least throughout the life of the organs of which they are a part. Other distinct and more or less differentiated regions sooner or later display a meristematic character. Among them are layers of cortex and phloem which may in time function as phellogen; and the pericycle of stem or root in which primordia of adventive roots commonly arise. Many other regions may become meristematic under the influence of appropriate stimuli.

All cells, then, originate as elements of a group which

is marked by the capacity of its cells to divide. It is conceivable that in such a group cells might be formed which, destined to become differentiated, can not themselves divide. However, so many cells in so many regions do divide under ordinary or special conditions, as for instance in consequence of wounding, that it can hardly be doubted that every plant cell at the beginning of its existence and for some time thereafter is capable of dividing.

That the power to divide may be lost in time is obvious. Senility doubtless results sooner or later in its loss. Structural difficulties, such as the development of thick walls, may hinder or inhibit division. It is often noted that cells whose walls have been markedly thickened ordinarily do not divide in response to a stimulus such as that of a wound. Not a few instances are reported, however, in which such cells (necessarily while still living), becoming dedifferentiated through the loss of their secondary wall layers and through other changes, proceed to divide. They return to a meristematic condition. This happens, for example, to the lignified cells of secondary xylem in stems of flax and of both secondary and primary xylem in stems of hemp (Schilling, 1923); and to the bast cells in roots of *Philodendron*, and to cells of the endodermis, thick-walled cortical cells, bast cells and vessels in the roots of several orchids (Bloch, 1926). Hence it seems that the power of division, inherent in each cell, may persist for a long time.

Granting this generally present capability, it remains to inquire as to the range of the structures whose development can result from the division of a given cell. In those plants, such as the filamentous algae and fungi and some of the simpler hepatics, whose cells remain substantially undifferentiated, it seems to be true that from any cell a complete new plant may arise.

In more massive individuals, such as gametophytes of mosses and the sporophytes of vascular plants, the condition is, as has been said, very different. In these, every cell, however highly differentiated it is destined to become, begins its existence in the meristematic condition.

In mosses, particularly, it is evident that very many of the cells of the gametophyte remain throughout their active life capable of developing into new plants. This capacity is manifested in part by the production in many species, on the most diverse parts of the plant, of vegetative reproductive structures of the nature of gemmae. Since some of the structures classed with gemmae are of the nature of abscised leaves, branches, or branch tips, this type of reproduction overlaps with the likewise widespread occurrence of proliferation from the most varied organs. Such proliferation in most cases begins with the outgrowth of a cell into a protonema—a stage preliminary to the production of leafy shoots. Sometimes the first outgrowth is a rhizoid; but since rhizoids and protonemata intergrade, and since either type of filament readily gives rise to the other, the distinction is not for present purposes important.

In some mosses proliferation from parts not specifically set apart as reproductive structures occurs without apparent external stimulation; the stem may bear a dense felt of rhizoids capable of developing protonemata and these in turn of producing leafy shoots. But more commonly rhizoids or protonemata are put forth in response to stimuli such as those of wounds or the removal of the part or organ concerned; sometimes in response to drying or to the aging or death of neighboring parts. Under these conditions protonemata grow from leaves or leaf fragments, fragments of stem or branches, even from isolated sex organs or paraphyses.

As Correns (1899) has pointed out, proliferation occurs not from all the cells of a particular organ. Often, on the contrary, the new development begins only in certain initials, which may be recognizable in advance. In the case of an organ of some thickness, as a stem, these initials are, almost necessarily, certain of the superficial cells. In a few species proliferation occurs from an interior region of the stem exposed by cutting, but here also only from the thin-walled cells of certain tissues. When to these facts is added the further one that in some

mosses, notably species of *Sphagnum*, proliferation has not been induced by any means tried, it is clear that the broad statement sometimes made that *any* cell of a moss gametophyte is capable of this type of regeneration is not proved. Correns, however, noted that in many cases the outgrowth and proliferation of particular cells are prevented by mechanical hindrances such as thickened walls. While, therefore, it is not demonstrated that every cell has the capacity to give rise to a new plant, neither is the absence of such potentiality demonstrated. It is probable that further experiment will show the possession of this power by at least a wider range of cells.

Also to be mentioned is the capacity of parts of wounded moss sporophytes to proliferate, giving rise to protonemata and through them to all the structures of the gametophytic generation. Here also there are differences among species; in some, such proliferation is readily induced, in others it has not proved possible of induction. These outgrowths, too, seem to come only from cells on the cut surface, and only from those of certain tissues.

In pteridophytes and seed plants, functional differences become more evident between types of meristematic cells, as manifested by the nature of the tissues to which, in usual course, they give rise. Apical meristems are commonly divided into fairly distinct regions, each regularly giving rise to particular primary tissues or tissue complexes. True cambium produces secondary xylem and phloem; phellogen produces cork and phelloderm.

On the other hand, it is often the case that, without apparent external stimulation, whole new organs with all their component tissues develop from a single meristematic region. An illustration is the frequent origin of lateral roots from the pericycle. Here the genotype of the pericyclic cells included all the tissue potentialities of a root. In some species, as in the horseradish (Lindner, 1938), a lateral root or a cutting therefrom can give rise to a bud which ultimately produces a new plant. In this case the genotype of the originating pericyclic cells

in the primary root evidently included all the potentialities of the race.

Buds borne on a stem which upon separation give rise to new plants are familiar. These buds, however, have arisen ordinarily from the apical meristem, and their formation as a rule involves each of the meristematic regions of the stem tip. The same is true of structures of the nature of bulbils, serving for vegetative propagation and analogous to the gemmae of bryophytes. In a different category belong adventive buds occurring commonly on stems, sometimes on roots as in the horseradish already cited, and resulting from a renewed meristematic activity of cells at and near the surface—cells of epidermis and cortex. A striking case of the regular occurrence of adventive buds on roots is that of *Pogonia* (Carlson, 1938). A bud appears at or near the tip of the root. If a little behind the root tip, its development involves a division of the differentiated cells of epidermis and cortex. After the new shoot has grown for a time it in turn forms adventive roots. New plants may develop, too, from buds formed on leaves. Hagemann (1932) lists 46 species belonging to 11 families (all angiosperms save one *Gnetum*) whose leaves, still attached to the parent plant and uninjured, bear buds.

Apart from such developments in the course of the regular life cycle, a similar production of new organs occurs in consequence of a variety of special causes. The new development is commonly the result of a stimulus applied from without; sometimes it arises in consequence of the aging and death of neighboring cells or tissues, or of the abscission of a leaf, bud or branch. An effective stimulus of external source is most frequently a wound, or a development, hormonal or other, consequent upon wounding. The resultant proliferation may involve cells adjacent to, or at some distance from, the wound.

Proliferation after wounding begins most frequently with the growth and division of cells beneath those which have been directly or indirectly killed by the injury. In an excised or otherwise wounded leaf (Wylie, 1930, 1931)

cells of all differentiated tissues beneath the wound except, apparently, the thick-walled cells of the vascular bundles, may be stimulated to divide. Following their division and that of their descendants, and the suberization of some of the outer cells, there is often (in dicotyledons) a development of a distinct phellogen. If meristematic activity continues beneath the phellogen, a thickened callus is formed. Differentiation of vascular and other tissues may occur within the callus. In some species buds arise from superficial layers of the callus, roots from its deeper-lying tissues. In *Crassula multicava* (McVeigh, 1938), both root and shoot arise from the epidermis near the wound. On excised scale leaves of *Lilium* (Walker, 1940), buds appear at some distance from the cut surface; leaf and stem primordia arise from a secondary meristem produced by the division of subepidermal cells; root primordia from interior cells adjacent to a vascular bundle. Separated leaves of several *Crassulaceae* produce adventive shoots and roots from already present meristems.

Wounded surfaces of stems, roots and immature fruits characteristically form calluses, as do those of leaves. Stems commonly, roots much less frequently, produce new shoots from calluses. Roots may develop from internal tissues of the wounded organ; if this does not occur, they are formed sooner or later by the new shoot. On stem cuttings the new adventive buds and roots sometimes appear at a distance from the wound; or buds may appear in one region, roots in another. The particular tissues of the wounded organ whose cells are stimulated to divide vary greatly from species to species.

Similar in their causal relations to the proliferations just mentioned, but different in their development, are growths already referred to of the nature of intumescences or galls. In some instances, as in certain interspecific *Crepis* and *Nicotiana* hybrids (Kostoff, 1934), tumorous growths occur spontaneously.—perhaps in these cases in consequence of an interaction between protoplasmic substances derived from the respective parents. The

better known and more frequent forms of overgrowth result from the bites and egg-deposition of insects, from infection by bacteria or fungi or from chemical stimulation. The varied tissue types that appear in these growths, as well as the frequent production from them of adventive roots and, especially in those forming witches' brooms, of adventive shoots, indicate something of the range not only, as previously suggested, of the genotype of the originating cell which gave rise to the plant, but also of the genotypes of those tissue cells which produced the overgrowth.

Stimulated growth occurs also from apical meristems. Since an apical stem meristem regularly gives rise to all the tissues of the stem, and since the stem may produce all the organs of the plant, growth from such a meristem, following for instance upon wounding, can supply no new evidence as to the genotypic possibilities of its cells. Different in its bearing is the evidence supplied by the culture of excised roots or root tips of various dicotyledons. In these cases, as illustrated by the tomato (White, 1936), growth and the differentiation of all primary tissues may continue indefinitely. Abundant branch roots are formed. But no interfascicular or cork cambium, and consequently no secondary tissues, are produced. No shoots are formed. It is true that roots of the same species, while attached to the parent plant, form cambia and secondary tissues—that is, their apical meristems ultimately give rise to all the elements of a complete root system. But in these, as in the majority of species studied, it has not been possible to demonstrate that from a root meristem can come all the structures of a complete plant. That the limitation here suggested is not universal is shown by the fact that the development of shoots from root cuttings is regular practice in the cultivation of a fair number of plants.

A comparable condition as to the cells of leaves is indicated by Hagemann's (1932) summary including his own extensive observations. He reports on the behavior of isolated leaves of 1,204 species of gymnosperms, dicotyledons and monocotyledons. Those of 289 species re-

generated both shoots and roots; those of 25 produced only shoots, which however in most instances themselves formed roots; leaves of 501 species produced only roots; and those of 389 formed no new structures whatever. Of 21 gymnosperms whose leaves were tested, those of six produced roots only; the others gave only negative results. In Schwarz's (1933) later study of 93 species, leaves of 8 produced shoots and roots; those of 38, roots only; those of 47, no new structures.

From the available evidence, fragmentary parts of which have here been cited, it is clear that, as has long been held, a large proportion of plant cells manifest, through the products of their division, the possession of a genotype equivalent to that of the race. But it is far from being demonstrated that all cells of all plants are in this sense totipotent. The opposite conception is suggested by the fact that, so far as experiment has shown, the cells of some tissues can give rise only to certain plant parts. Conspicuously, in the majority of angiosperms tested, leaves, roots and root meristems seem unable to regenerate complete plants. Doubtless the power of proliferation may by improved methods be shown to be more wide-spread. But for the present it must be admitted that the comprehensiveness of the genotype seems to vary as between the cells of different tissues, of different organs, and even more as between cells of corresponding tissues in different species.

This conclusion, if it becomes a conclusion, contradicts the expectation derived from the general uniformity of nuclear structure throughout the life of a plant. There would seem to remain as the only possible explanation of the apparent facts the occurrence of genetically effective cytoplasmic differences between the cells of different tissues or organs. Much evidence indicates that related species may differ significantly in cytoplasmic constitution, and that this constitution favors or inhibits the expression of particular genic potentialities. The cytoplasm of a cell, then, in some measure helps to determine its genotype. The possibility is suggested that in the course of ontogeny cytoplasmic differences may appear,

one consequence being that, while the ability to divide persists, the genotypic capacity of the cells of particular regions is limited. In the absence of more positive evidence, this possibility remains for the present nothing more.

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INDUCED CHROMOSOMAL CHANGES AND THEIR SIGNIFICANCE IN GROWTH AND DEVELOPMENT

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Of all the structures of the cell the chromosomes have unquestionably attracted the major attention of investigators principally because it was early demonstrated that they are in some way associated with heredity. The idea that the hereditary units were contained in the chromosomes had been presented in the writings of Weismann, Boveri, Strasburger and Hertwig before the rediscovery of Mendel's Laws in 1900, and was developed further by Cannon and especially by Sutton.

Since then, overwhelming evidence has accumulated in support of the chromosome theory of heredity. Boveri, and especially Morgan and Bridges, have proved that every chromosome is an individual in the sense that it carries a definite complex of genes. The theory of the linear arrangement of the genes within the chromosomes, formulated by Sturtevant, has become a fundamental concept of modern genetics and cytology.

Every individual, race or species has a relatively constant genotype, that is, the sum total of the hereditary potentialities, inherited from its ancestors. The stability of the genotype is responsible for the persistence of the visible traits of the organism. The genes, however, are carried in the chromosomes and every organism is characterized by having a definite number of chromosomes, and every chromosome bears a definite complex of genes arranged in a fixed linear order. These stable structural units constitute the karyotype. A chromosome, as well as a gene, is a self-duplicating body and is potentially able to duplicate itself exactly an indefinite number of times. Nevertheless, the constancy of the karyotype, like that of the genotype, is subject to certain variations. The chromosome structure is subject to change, and an altered

chromosome, like a mutant gene, may retain the ability of self-duplication indefinitely.

The visible appearance of the chromosomes is to a certain extent determined by the genes which they carry. Navashin has described changes in the morphology of chromosomes following hybridization in species of *Crepis*, and Mann and Frost have found similar changes in the chromosomes of hybrids between strains of *Matthiola incana* where the chromosomes differ in a single gene. The nature of these changes is a matter of conjecture, but they appear to be a result of gene mutation. As a rule, however, gene mutation appears not to produce any visible alteration in the appearance of the chromosome in which it occurs.

In addition to alterations resulting from genic changes, there are various chromosome alterations of an entirely different kind. Chromosomes, or parts of them, may be reduplicated or lost, thus resulting in individuals with an excess or a deficiency of certain genes. These alterations may be distinguished under two main heads: (1) numerical changes and (2) structural changes.

Numerical changes may involve a change in the number of sets of chromosomes present in the nucleus, as in haploid or polyploid organisms, or it may involve a change in the number of separate chromosomes in a set, as in monosomic or polysomic organisms, without changing the gene content or arrangement within the individual chromosomes.

Structural changes, affecting the gene content of the chromosomes may be of two general types: (1) changes due to the loss or reduplication of some of the genes, thus resulting in a deficiency (deletion) or a duplication, and (2) changes due to an alteration of the normal arrangement of the genes as a result of a translocation or of an inversion.

All these types of changes may occur spontaneously, that is to say, with no apparent cause, but they may also be induced experimentally in a number of ways. Among the agencies which have been used are x-rays, radium,

temperature variations and various chemicals. Any of these agencies may produce gene mutations, chromosome changes, or both.

The discovery of X-radiation by Röntgen was followed by examination of its effect on plant and animal cells, as was the isolation of radium by the Curies three years later. These tests on various organisms made it apparent that exposures to X-radiation and radioactive substances produced similar biological effects. Certain of these early studies of the effects of x-rays and radium indicated that marked alterations were effected in the nucleus, especially during divisions. Striking chromosomal disruptions were recorded as early as 1904, with the most marked effects being observed after heavy dosages.

With the rediscovery of Mendel's principles special interest attached to spontaneous mutations. The study of the inheritance of character contrasts in a variety of organisms was stimulated. All morphological variations found in a given species were considered, and because they were comparatively rare and difficult to obtain special attempts were made to induce mutation artificially. Many kinds of organisms in different stages of development were subjected to various kinds and combinations of external stimuli, such as centrifuging, mechanical injury, temperature changes, various chemicals, etc., with varied success, but for a time no method or agent could be found which would produce mutation at the will of the experimenter. High frequency radiation had also been utilized and mutations induced, but the experiments had been on so small a scale that the effective nature of these radiations was not then apparent. But the extensive series of experiments which Stein initiated in the early nineteen-twenties on *Antirrhinum*, in an effort to determine the effects of radiation, produced a variety of aberrant forms, some of which were considered to be mutant types resulting from the treatment.

However, the first successful attempt to induce mutation in controlled experiments was made by Muller, using x-rays on *Drosophila*. At the same time Stadler was

working independently on barley and maize, and his results, published almost simultaneously, were comparable to and confirmatory of those secured by Muller. Since then much work has been done on both plants and animals and a large body of data secured which shows that the effect of radiation, from both x-rays and radium, is a general increase in the mutation rate, including both gene mutations and chromosome aberrations. There appears to be no specificity in the response, and it is not yet possible directly to obtain the reaction of any particular allelomorph or chromosome locus, although in some species breakage of the chromosomes seems to occur most frequently at or near the centromere, near the ends or in some other localized region.

The types of mutations induced by radiation are of the same general nature as those occurring spontaneously. In fact, many of the induced types were known previously. It is more difficult to decide whether the different types occur with the same relative frequencies under irradiation as they do spontaneously, because, at first sight, one is impressed with the high percentage of lethals in x-rayed flies (over 90 per cent. of all mutations produced), but when spontaneous mutations are critically investigated by the same methods, it is found that here also lethals occur in about the same proportions.

The more conspicuous alterations induced by radiation are chromosomal in nature. The smaller, viable and genetically most significant of these are the transverse fractures of one or more chromosomes which result in fragments. Internal rearrangements, losses or additions of chromosomal material, usually follow. Such alterations of the chromosomes, involving especially inversions and translocations, may be retained through succeeding mitotic and meiotic divisions in the treated material, and in later generations, unless the alteration is of such nature that normal cell or nuclear division is not possible. In the latter case, an approximate division may occur, resulting in unequal apportionment of nuclear material to the daughter cells, the products being either inviable and

incapable of survival or viable and hence leading to the formation of a new genom. A series of variant chromosome forms may thus be established.

Irradiation does not often result in an increase in number of chromosomes, but there are several agencies by means of which numerical changes may be brought about. The effects of chloral hydrate have been known since the work of Nemeec, followed especially by that of Sakamura and van Regemorter. Their investigations showed that chromosomes in root tip cells subjected to the influence of chloral hydrate behave with an irregularity which varies with the strength of the dose. In spite of the aberrant types of division induced, some of which result in doubling the number of chromosomes, no one has reported the production of a polyploid plant from its use.

Although it had been observed earlier that wide fluctuations of temperature might induce chromosome doubling in plant cells, practical results were not obtained until Randolph subjected the zygotes of maize to a temperature just below the maximum for their survival at the time when they were to divide and begin embryo formation. By this treatment he secured a few seeds which developed into tetraploid plants.

The cytological effects of temperature treatments have been studied by Sax and Dermen, who found that temperature changes, either high or low, caused a clumping of the chromosomes at the metaphase in the microspores. At either first or second meiotic division they may fail to separate, remain aggregated and form a single nucleus by going into the interphase (resting stage), shortly after the plants were subjected to the treatment. If the plants were then returned to normal temperatures the affected cells resumed development, but contained a double number of chromosomes. One meiotic division is thus affected by the temperature treatment and this results in the production of two diploid microspores instead of the four monoploid microspores normally developed. Only by a close succession of temperature treatments may both meiotic divisions be prevented and tetraploid micro-

spores be produced. Many irregularities were observed, among them being fragmentation, translocations and inversions, as well as asynapsis; these irregularities are similar to those induced by radiation, certain chemicals, and to those which occur spontaneously.

Chloroform has been reported, in at least one instance, to induce chromosome doubling. It probably induced ameiosis, thus leading to doubling of the chromosomes and the production of a fertile amphidiploid. This agent, like ether, has apparently been little used and its possibilities for inducing numerical changes in chromosomes are as yet largely unknown.

Colchicine has come into prominence during the last few years and has attracted considerable attention because of its rather specific action upon mitosis and meiosis, particularly in plants. Colchicine occurs naturally in the Autumn Crocus, *Colchicum autumnale*, and has been long known as a poison and as a specific for gout. Dustin of Brussels appears to have been the first to report its action in arresting nuclear division. Allen and his associates at the Yale Medical School used colchicine in experiments on animal tissues and found that it influenced mitotic activity in them. Eigsti became interested in the possible cytological effects of colchicine on plant cells and he, Blakeslee, Blakeslee and Avery, and Nebel were among the first to publish accounts of the actual production of polyploidy (tetraploidy) in plants and to call attention to the significance of the substance as an agent for inducing chromosome doubling in sterile species hybrids. Since then a number of investigators have done a large amount of work on plants, and their results are well known. There is general agreement among cytologists as to the mechanism involved.

In mitosis colchicine inactivates the spindle mechanism and delays the division of the centromeres. Prophase stages ordinarily show no irregularities. The nuclear membrane disappears but no spindle develops and the condensed chromosomes, which show relational coiling

between their chromatids, form an irregular metaphase plate.

After a few hours the centromeres divide, but the daughter chromosomes, in the absence of a spindle, do not pass to the poles, but remain in a group which occupies the approximate original volume of the parent nucleus. A nuclear membrane forms about them and the number of chromosomes becomes doubled as a result of the longitudinal division. Cells which originally possessed a diploid complement of chromosomes become tetraploid. Exposures to colchicine of a few minutes to one hour effect only one mitosis in a given cell, while longer exposure may influence more than one, each doubling the chromosome number.

The spindle mechanism begins to recover 12 to 24 hours after exposure, and in 36 to 48 hours the mitoses again run a normal course in both diploid and polyploid nuclei. Until recovery is complete, however, an intermediate stage is found during which multipolar and other aberrant types of spindles occur.

Although colchicine has been used chiefly in producing somatic doubling, it has also been used on flowering stalks of certain plants. Walker found it suppressed the spindle mechanism entirely during one or both meiotic divisions and sometimes inhibited cell division after the first meiotic division. Diploid and tetraploid microspores and pollen grains were commonly produced and occasionally octoploid and hexaploid microspores were found. Octoploid microspores may have resulted from the suppression of either the premeiotic or postmeiotic mitosis and both meiotic divisions. Partial suppression of the spindle mechanism resulted in the formation of triads, pentads, hexads and octads.

If the second meiotic division only is affected by the colchicine, dyads containing the diploid number of chromosomes are produced. Divisions in microspore nuclei are affected in a similar manner to those in root tip cells.

In general it has been found that colchicine does not produce deleterious effects on the chromosomes, such as

fragmentation, translocation and inversion, but Eigsti has reported "chromosome breakage" in pollen tubes of *Polygonatum commutatum* treated with colchicine, showing that possibly "colchicine induces variations other than polyploid changes." Similar effects have not been reported by other investigators.

Aneuploidy, which appears to result from a partial instead of a total arrest of centromere division as the colchicine begins to become effective or as its effect is waning, is of frequent occurrence.

Colchicine appears to influence the most active stages of nuclear division by bringing about a delay in the division of the centromeres and inhibiting spindle development and hence anaphasic movement of the chromosomes.

Shortly after the use of colchicine for inducing polyploidy had been announced, Schmuck, Navashin and Kostoff, separately, in about a half dozen papers, announced that acenaphthene produces an effect on nuclear ploidy had been announced, Schmuck, Navashin, and Kostoff reported that acenaphthene prevented the anaphase separation of chromosomes and thus causes chromosome doubling. It appears to inhibit spindle formation in much the same manner as colchicine, but after more than one doubling of the number had occurred the chromosomes became separated into groups which were divided off by cell walls. Several other workers have failed to duplicate Kostoff's results, but a recent paper by Levan gives a critical comparison of the effects of both colchicine and acenaphthene on *Allium fistulosum* and the effect of acenaphthene on *Colchicum autumnale* from which colchicine is derived and which is not affected by it. Levan found that acenaphthene is much slower in action upon Allium, perhaps a thousand times, than colchicine and in its earlier action it may involve only a part of the spindle and of the chromosomes and thus produce irregularities in divisions which result in aneuploidy. When its effect has become maximum, however, its action is similar to that of colchicine and results in chromosome doubling in a similar manner. In general its effect is not so cer-

tain as that of colchicine, but it is less expensive and probably much safer to handle. Yet much more work will need to be done with it because of its slower action and possibly its more variable effects before it may come into general use as a polyploidizing agent.

There are indications that certain of the growth-regulating substances may induce polyploidy. Greenleaf applied indoleacetic acid to the cut surfaces of decapitated stems of *Nicotiana* and secured a number of tetraploid and octoploid shoots among the normal diploids. The tomato, a fairly close relative of *Nicotiana*, will produce tetraploid shoots when merely decapitated if the cut surface is protected against drying. It is possible that the indoleacetic acid may serve as the excitant for callus formation in *Nicotiana*, and once this is developed, the same conditions or factors which operate to cause chromosome doubling, or quadrupling, in the tomato may become operative in *Nicotiana*. There is no evidence from experiments on bean, *Iresine*, *Lilium* and tomato that indoleacetic acid induces polyploidy.

Two recent papers have presented evidence which indicates that others of the growth-regulating substances may induce chromosome doubling.

Levan subjected roots of *Allium* to solutions of naphthalene-1-acetic acid and indole-3-butyric acid at concentrations of from 1 to 10 parts per million. Both substances and both concentrations effectively inhibit root elongation, and result in a marked swelling just above the apical growing point. Cytological examination showed that the cells of the swollen region nearest the root apex contained the diploid number of chromosomes ($2n = 16$), while farther back from the apex the majority of the cells showed 32 chromosomes. Still more distant from the apex cells with 64 chromosomes predominated. The mechanism involved in the doubling process was reported to be entirely different to that which is operative when colchicine is used. Colchicine interferes with or inhibits the anaphasic movement of the chromosomes, while these two growth-regulating substances effect doubling dur-

ing the resting stage. In the early prophases of mitosis the chromosomes show a quadripartite instead of the usual bipartite structure, with a single centromere holding the chromatids in a single association. At late prophase the nuclear membrane disappears and the 16 quadripartite chromosomes are arranged in an equatorial plate with the four chromatids lying parallel to one another. The spindle develops and functions normally. The centromeres divide to produce 16 pairs of chromosomes which remain in a paired state for a short time. Immediately afterward the centromeres divide once more and this is followed by anaphasic movement of the chromosomes, with 32 passing to each pole, thus resulting in the production of tetraploid nuclei.

Dermen applied naphthalene acetic acid in lanolin paste to internodes of the bean and produced tumorlike growths on them. Cytological study of the tumors showed them to contain numerous tetraploid, a few octoploid and occasional 16-ploid cells. These were found in the cortex, endodermal region, phloem, medullary rays and pith of the treated stems, and also in the cortex and endodermal region of the root primordia resulting from the treatment. Polyploid cells were not observed in the cambium of the stems or the procambial region of the root primordia, but, in a few instances, were seen in the growing points of the root primordia. Doubling appeared to take place during the resting stage of the nuclei, as described by Levan.

Through the use of sulfanilamide and two or three other sulfa-compounds Traub has reported the induction of chromosome doubling. Onion bulbs were placed with their bases in water for two days, transferred to 0.25 and 0.5 per cent. solutions of sulfanilamide for two days, and then back to water for two days before fixing the roots for study. Hypertrophy became evident in the roots within 24 hours after placing them in the sulfanilamide solution, and microscopic study showed various abnormalities, such as tetraploid cells, binucleate cells and dumbbell shaped nuclei. Evidently sulfanilamide and

other sulfa-compounds may induce polyploidy and other cell and nuclear aberrations, but how they bring about this effect is not discussed by Traub.

With the possible exception of indoleacetic acid, none of the growth-regulating substances so far used has induced polyploidy in tissue regions likely to produce buds. On the contrary, the affected cells have been found in regions destined to early death and disintegration, and that these cells may subsequently lead to the production of polyploid plants seems improbable.

There are doubtless other agencies and substances by means of which chromosome doubling and other types of changes may be induced, but those discussed above will serve to illustrate the range of changes which are known to be induced.

Chromosomal changes, like gene mutations, were first recognized from their spontaneous occurrence. De Vries described certain spontaneous mutants in *Oenothera*, some of which were later shown to be the result of chromosomal changes. The first deficiencies, duplications and translocations, observed by Bridges, and inversions, reported by Sturtevant, were detected in *Drosophila melanogaster*, and either arose spontaneously in laboratory cultures or else were present in the natural populations from which the experimental flies originated. Polysomics, monosomics, polyploids, haploids and translocations were observed in *Datura stramonium* by Blakeslee.

Structural changes appear to involve more profound processes than numerical ones, since the former requires breakage of the chromosomes and reconstitution of new ones from the fragments. Two hypotheses have been advanced to explain the mechanism involved. One of them, known as the "contact hypothesis," assumes that chromosomes occasionally undergo "illegitimate crossing over" between non-homologous sections. According to this view, the chromosome breakage and reunion of the fragments occur simultaneously and are parts of the same process. The other view, known as the "breakage first"

hypothesis, is that chromosomes undergo fragmentation, owing perhaps to the destruction of a gene or genes somewhere in the linear series or to a severance of an intergenic connection. Following this the fragments may remain free, become reattached to establish the original gene arrangement or may unite with other fragments to form new arrangements. Recent experiments indicate that the "breakage first" hypothesis is probably the correct one.

Early studies on chromosomal aberrations induced by x-rays showed that only those rearranged chromosomes having a single centromere are capable of survival. Chromosomes having no centromere or more than one are formed, but are later eliminated because it is not possible for them to be transmitted regularly during nuclear divisions.

Associated with chromosome rearrangement are translocations and inversions. Translocations appear to occur much more frequently among plants than animals, especially the reciprocal type, which is known to occur in numerous plant species.

Translocation heterozygotes usually, but not invariably, produce some gametes with duplications and deficiencies for certain chromosome sections, and these may amount to 50 per cent. or more of the total. Since duplications and deficiencies are frequently lethal, the unbalanced types are lost, and the reproductive potential of translocation heterozygotes are lowered. If, however, the translocation becomes homozygous the chance for survival is greatly increased. It seems highly probable that losses, duplications and translocations have played an important role in the production of widely different chromosome complements and genotypes and therefore in the origin of fertile and stable types to which specific rank has been assigned.

Inversion is another consequence of chromosome breakage and reassembly. The portion inverted may include one end, an interstitial section or more regions. More organisms, among both plants and animals, containing

inversions are constantly being found, and there can be little doubt that through inversion evolution of changed chromosomal types is wide-spread in nature and probably exceeds translocation in importance in bringing about racial differences which may lead to species differentiation.

The differentiation of races and species by gene mutation and structural alterations of the chromosomes is a slow and cumulative process. But alongside the slow method there exists in nature a quite distinct one which results in the sudden emergence of a new species from preexisting ones. The slow method occurs throughout the entire living world, and may in this sense be considered the general one, while the sudden origin of new species is confined mainly to some large groups in the plant kingdom. The latter method is connected with a multiplication of the chromosomes, and occurs mostly as a result of hybridization of two previously existing species. The chromosome complement is doubled in the hybrid, and the resulting allotetraploid possesses all the genes which were present in the two parental species and no new genes. Since the ancestral species may continue to exist side by side with the new, the total diversity is increased by this process.

The fertility of an organism is strikingly affected by polyploidy. If the ancestral organism is a fertile species the polyploid derivative will be partially sterile, owing in part, to multivalent associations of chromosomes and their irregular segregation. If, on the other hand, the diploid organism is a sterile hybrid the polyploid (allotetraploid) derived from it is generally fully fertile. Thus two general types of polyploids occur, autopolyploids, which contain more than two complete sets of chromosomes derived from a single species, and allopolyploids, which result from chromosome doubling in a hybrid. These are general definitions and it is not always possible to determine whether an organism is auto- or allopolyploid. In some instances both conditions may exist in the same organism, and both frequently occur in

the same complex of related species. But it is generally accepted by most cytologists and geneticists that allopolyploidy has played the much more important role in species formation and evolution among seed plants.

Polyploidy is relatively rare among animals and appears to have been of little evolutionary significance in this kingdom. It is common and wide-spread among plants, particularly in the angiosperms, in which Muntzing has estimated that more than half the species occurring in nature are polyploids. This estimate may perhaps be too high, but certainly it is not far wrong.

Cytogenetic research has been greatly stimulated by the discovery of substances and methods by means of which all the chromosomal changes, including gene mutations, known to occur spontaneously may now be induced experimentally. Through treatment with colchicine or some other of the agencies which induce chromosome doubling a number of allopolyploids have been obtained in recent years from both interspecific and intergeneric crosses. Thus a greater amount and range of material and detailed evidence has been made available for study with the result that a clearer understanding of the processes involved in racial and species differentiation has been obtained.

Many of the induced alterations are of doubtful value to the organism, some are harmful, and even lethal, but much evidence indicates that the same is true of spontaneous variations normally occurring in wild species. The perpetuation of variation does not necessarily depend on whether it is beneficial or not. The size of the population involved and the environmental conditions surrounding it appear to be of more importance than the nature of the alteration in determining its survival.

Finally, the ease and readiness with which polyploidy may be induced should justify a confidence in the possibilities which lie ahead of plant breeders for producing not only new autopolyploids with enhanced values, but new allopolyploids in which are combined the desirable characteristics of two or more existing varieties or species.

THE PROBLEM OF INTERNAL DIFFERENTIATION IN PLANTS

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ONTOGENY is typically a progress toward greater diversity between the parts of an organism. Differences in the rate and direction of growth produce the specific external forms of organ and body, and differences in the size, shape and character of its cells result in equally specific internal differentness. The factors which produce this organic differentiation are still very obscure, but they must evidently be closely involved with the general problems of development and morphogenesis. The present paper is an attempt to analyze certain of the problems of differentiation which to-day confront the student of plant histology.

Growth in the higher plants results from the multiplication of cells at embryonic regions or meristems. Such cells are relatively uniform in appearance and are commonly spoken of as "undifferentiated." Very early in the developmental process, however, usually before cell multiplication has ceased, differences appear among them and soon those groups of specialized cells known as tissues make their appearance. The differences between these are profound. Cells from the pith, the xylem and the epidermis are as unlike as cells can well be, but they all originate from the essentially uniform cells of the embryonic regions. Here is one of the great paradoxes of biology: each of these cells remains genetically equivalent to every other one and if isolated under proper conditions could doubtless produce the entire organism, yet each will nevertheless develop in a very specific fashion and become different from most of its neighbors. Why, we may ask, should cells fundamentally alike thus diverge so radically? This origin of histological diversity in the midst of genetical identity is the fundamental problem of differentiation.

Differences between tissues must first arise as differences between cells, so that our problem is in large measure that of the origin of cellular differentiation. Cells may differ in size, form, wall development, physiological behavior and other respects. The pulp cell of a watermelon may be 100,000 times as large as an epidermal cell and a million times as large as a meristematic cell. A tracheid, fifty times as long as wide, contrasts sharply with an isodiametric pith cell. A heavily lignified stone cell and one from delicate storage parenchyma represent extremes in wall difference. Physiological diversity is as great as structural, though often more difficult to recognize. All this is the familiar domain of comparative histology.

These differences between cells do not arise at random, but show a very definite arrangement. This may be simple, as the gradient from large to small cells commonly found between the center and the periphery of the fundamental tissue in the axis of shoots and fruits. More complex is the origin of vascular tissue in the shoot meristem, about which confusion has long existed but which is now being cleared up by Wetmore and Esau, who have established the relation of differentiating provascular elements to older vascular tissue and to the leaf primordia. Even the orderly origin of particular tissues from specific cell layers at the meristem itself has recently been shown in *Datura* by Satina and Blakeslee, who were able to trace the developmental history of each layer by a study of periclinal chimeras produced by colchicine.

The factors responsible for the origin of these cellular differences have long been objectives of students of plant morphogenesis, and a considerable body of knowledge about them has been acquired. Some of the more important aspects of this will be reviewed briefly here under a number of categories.

DURATION OF CELL DIVISION

One of the first differences which appear among embry-

onic cells is in the duration of cell division. Thus it has been shown that the marked increase in cell size toward the center of the cucurbit fruit is due chiefly to the fact that division ceases first (and rapid vacuolation thus begins first) in the center and then progressively toward the outside. The contrast is often much sharper than this, as in the giant cells of the pericarp of *Capsicum* which owe their great size to the fact that in them division stops early but cell growth keeps pace with the continued multiplication of adjacent cells. In the same way, elongated cells, as in the coleoptile epidermis or provascular tissue, acquire this shape because they stop dividing at an early stage in axis extension but expand lengthwise as fast as the adjacent tissue grows by continued transverse divisions.

Factors which control cell division therefore have an important rôle in differentiation as well as in growth. So many of these have been reported that here we can do little more than enumerate the more important of them. The attainment of a given cell size and thus of a specific surface-volume relation; the attainment of a particular size relation between nucleus and cytoplasm; changes in cytoplasmic viscosity; certain radiations; and various chemical substances such as sulfhydryl, nucleic acid, auxin, wound hormones and many others have all been suggested as playing important parts in the induction of mitosis. This field of developmental physiology is particularly active to-day and is providing important information as to various aspects of cellular differentiation.

PLANE OF CELL DIVISION

The position of the new cell wall is also important in producing cellular diversity. Certain cell size differences are due to unequal division, for example, between sieve tube and companion cell. The orientation of the wall is of particular importance. Thus absence of periclinal divisions in the epidermis is an essential distinction be-

tween it and the underlying tissue. The whole mechanism of growth by an apical cell is dependent upon a regular sequence of divisional planes. The origin of a ray initial in the cambium results from a small cell cut out of the side of a cambial initial. Such examples could be multiplied almost indefinitely.

Many of the factors concerned in this aspect of differentiation are also known. The plane of division of the egg of *Fucus* has been found to be affected by the direction of an electric current and of light, and by gradients in hydrogen-ion concentration, temperature and various chemical substances. In more complex tissues division has also been shown to occur at right angles to the direction of light and of hormonal diffusion and parallel to mechanical pressure. In our laboratory it has recently been shown that the position of the new wall is markedly affected by partition walls in adjacent cells. In very liquid cells surface tension has been thought to play an important part, though recent work casts some doubt on this conclusion.

CELL GROWTH

The size and shape of a cell are often determined by its neighbors or other external influences, but internal factors also play a part. In the cucurbit fruit the cells of the innermost tissues grow more rapidly, even during the period of cell division, than the outer ones, and a part of their ultimate size differences are due to this fact. Such growth differences are more conspicuous where cells are free to expand, as in trichomes.

Unequal cell growth often results in autonomous shape changes. Thus the localized growth of a root-hair cell profoundly changes the cell form as the hair develops. Idioblasts and similar cells partly free from surrounding pressure produce highly specific forms. Even in tissue masses, growth is often unequal, notably in such cases as rib meristems where cells of the fundamental tissues during vacuolation expand much more parallel with the axis than at right angles to it.

Some of these differences, as in the case of the root hair, are clearly preceded by local changes in the character of the wall which result in greater expansion at certain points than at others. Possibly the polarized expansion of axis cells may also be related to differences in the character of the longitudinal and the transverse walls. The many cases where single cells or unicellular organisms with extremely thin walls possess constant and highly specific forms suggest that these forms may be due to factors in the cytoplasm, such as a characteristic orientation of molecules or micellae. Even those modifications of the wall which result in shape changes probably take their origin in the cytoplasm.

THE CELL WALL

Differences in the character of the wall are so familiar that they hardly need enumeration. They constitute the most conspicuous aspect of cellular differentiation. Here, too, we are acquainted with some of the factors involved. Thus the degree of lignification is reduced by high humidity and increased by dryness. Suberization and cutinization often accompany the exposure of cell surfaces to the air and are presumably induced by oxygen. Indeed, Priestley's conception of the factors controlling meristematic differentiation is based largely on the idea of changes in the cell wall induced by exposure to air as intercellular spaces appear. Differentiation of an endodermal layer with its characteristic wall has been shown to be checked by light. Mechanical forces, notably lateral strain and swaying, induce the formation of collenchymatous wall thickening in cells which would otherwise remain parenchyma. Stimulation of metabolic activity, as by wounding, will often result in increased wall thickness. Our knowledge of the chemistry and physics of many wall constituents is far from complete, but we are beginning to understand some of the factors which modify their development.

NUCLEAR DIFFERENTIATION

A hitherto unsuspected aspect of differentiation has recently been brought to light with the discovery that many mature cells do not possess the basic chromosome number of the embryo or meristems but a multiple of it. This somatic polyploidy may sometimes be observed directly, as in roots of spinach, where at some distance back of the tip but in a region still meristematic a considerable number of tetraploid figures begin to appear and rows of tetraploid cells are established. In many other forms this change takes place after the last division but can be demonstrated by inducing mature cells to divide through the agency of various growth substances, and it often occurs in traumatic and tumorous tissue. Cells with 4, 8, 16 or even 32 times the haploid chromosome number have been reported by Geitler, Grafl, Gentcheff and Gustafson, and others. It is significant that somatic polyploidy does not occur at random but distinguishes specific tissues. Cell size tends to be roughly proportional to chromosome number, and polyploidy is thus directly concerned with some of the differentiation here, perhaps with much of it. In view of the many instances where polyploidy is known to produce qualitative as well as quantitative effects, somatic polyploidy may have an importance in other aspects of differentiation. It has been found so frequently that it must now be regarded as a normal concomitant of differentiation in many plants.

These various factors must evidently operate in such a way that at some point they produce a difference between two sister cells, for any instance of differentiation must evidently start with a division from which the two resulting cells are unlike, either from the beginning or in their ultimate fate. Our problem thus resolves itself primarily into that of differential cell division. Many of these may be observed. They are common, of course, in any indeterminate meristem where one cell remains embryonic and the other does not, as with a cambium initial. In forms which grow by an apical cell, such as the liverworts

and many pteridophytes, developmental progress is so regular that particular tissues can be traced back directly to differential divisions. Thus in *Selaginella* the cell arising from the apical one produces two daughter cells by periclinal division, the outer becoming ancestral to the epidermis and cortex and the inner to the endodermis and vascular cylinder. Such early parcelling out of fates at particular divisions is a familiar feature of the development of many plants. Other differential divisions are relatively late, as in the ontogeny of the stomatal apparatus in such plants as *Sedum*, where the smaller of two daughter cells in the developing epidermis gives rise by a series of divisions to a group of cells one of which is ancestral to the two guard cells. In some cases the visibly differential division is the last one. A good example is the development of root hairs in certain grasses. Here the final division of many surface cells of the root typically results in a smaller, more deeply cytoplasmic cell, the trichoblast, toward the apex, and a larger cell toward the base. The former produces a root hair, but the latter does not. This difference can be seen to begin in the mother cell itself before division, for in the apical end of this cell the cytoplasm is markedly more dense. Here is differentiation reduced to its lowest terms.

Such examples as these, where two diverse courses of development are in some way set apart at a given cell division—and presumably foreshadowed in the mother cell itself—are by no means infrequent, and many similar ones will occur to the histologist. In other cases of differentiation, however, particularly those arising in the embryonic stages of determinate organs like the leaf and fruit, it is often difficult to distinguish just the division where two divergent cell lineages separate, and even if this is found the sister cells and sometimes their immediate descendants may at first be very similar. Sometimes a physiological difference may precede a visible structural one, as in the case reported by Helm where the provascular cells in the apical meristem stain differently

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from the rest before any other differences between them and their neighbors are apparent. In many cases, indeed, a physiological difference is the only one which can be detected, as in cells with different pigmentation. Here it is often possible to show that a colored spot on a petal, for example, ultimately goes back to a single pigmented cell, which must have arisen from a differential division. The study of cell lineages of this sort has been facilitated by the occurrence of recognizably different genetic changes in a cell group which must have originated from a single cell. This may have been a gene mutation, as in the petal spots of *Delphinium ajacis* reported by Demerec; a chromosome change, as in the maize endosperm spots studied by Jones or in the leaf spots reported by Miss McClintock; or the result of somatic segregation, as in the stripes and spots occurring in various heterozygous plants, where an occasional division fails to partition the genic complement equally. Among the higher plants with their complex and massive meristems of apparently similar cells, it is impossible to trace back a cellular pedigree to the beginning, as can be done with the simpler forms growing by apical cells, but even in the angiosperms evidence is now accumulating that a comparatively small number of cells are ancestral to the whole axis. A careful study of the meristems of shoot and root certainly gives the impression that even with all their complexity, development involves a regular series of progressive divisions whereby a demarcation of the various organs and tissues is established from a very early stage. Incidentally, these facts all emphasize the great importance of the cellular unit in these processes, and support the view that its developmental significance is greater for differentiation than for growth. Growth could occur without cellular organization, but differentiation would be extremely difficult.

It is therefore clear that students of plant morphogenesis are possessed of a substantial body of knowledge with regard to the origin of those differences between

cells which arise during the process of development and as to the factors which are responsible for them. Most of the ultimate protoplasmic mechanisms involved are still unknown, but many of the differences observed in normal development can be reproduced by experimental means. It might appear from a rehearsal of these facts that we are coming close to an understanding of the phenomena of differentiation. Such a conclusion is far from the truth. The ultimate problem, of course, is not as to what cellular changes take place or what factors can be shown to produce them but as to why these various differences originate at just the particular places and times which will lead to the orderly development of an organism and not to a chaos. This is still the enigma of development. It is not cellular differentiation as such which eludes us, but the orderly control of the process.

Here the student of experimentally controlled development comes powerfully to the aid of the descriptive histologist and embryologist. What will happen, he asks, to this beautifully ordered course of progressive differentiation if one interferes with it artificially by changing the external conditions by wounding, or by excising a part of the growing structure? The answer to this question should certainly throw much light on the problem of developmental control, and experimental techniques have for many years proven exceedingly valuable. They have been explored much more fully by our zoological colleagues, who have long been accumulating a very great body of evidence showing how much wider are the capacities for differentiation which most embryonic cells possess than the particular one which they display under a given set of conditions. The advantages for such studies of the isolated and easily manipulated animal embryo are great and unfortunately can not be duplicated with most plant material. Some work has indeed been done, particularly with various lower plants, but so far without many results which can readily be carried over to the higher ones. Students of even the more complex plant types, however, have

an advantage which they have not yet capitalized to the full—the great plasticity of many plant organs and the remarkable capacity for the restoration of lost parts, for regeneration in the broad sense, which most plants display and which can be studied experimentally in the readily accessible structures arising from persistently embryonic meristems. It is often possible to re-route the developmental process, so to speak, and thus to make significant contributions to the problem of the orderly control of histological differentiation.

The results already accumulated by students of experimental morphology, from Vöchting's day to the present, are important and stimulating and deserve to be carefully examined and widely extended. There is opportunity in the present paper merely to summarize certain of the more general conclusions which bear on the problem of differentiation.

It has been established that differentiation in many cases is far from a regular and determined parcelling out of cell fates but that many cells remain for some time in a state of equilibrium which may be tipped one way or another by environmental factors. In some plants, for example, the determination as to whether or not a cell will form a root hair is irrevocably laid down at the last division. In many other species, however, *all* superficial cells retain this potency, and their ultimate differentiation depends on various external conditions. That such indeterminate differentiation is not rare is indicated by the ease with which certain characters can be modified by environmental agencies. Here the problem of differentiation meets those of potency, competence and reactivity about which nowadays we hear so much.

A study of the more drastic environmental changes which lead to regeneration has yielded two major results. One is the fact that many cells, far along the road toward complete differentiation, may under an appropriate stimulus actually *dedifferentiate*, losing the specific characteristics of wall and contents which they had acquired and

returning essentially to an embryonic condition. From this, under the exigencies of their newly established relations in the regenerating structure, they may *redifferentiate* along an entirely new path and to an entirely different end from that which they originally had attained. It is in a study of such radical diversions of the course of differentiation under controlled experimental conditions that we can perhaps approach most nearly to an understanding of the process itself. Particularly significant are the early stages following the experimental excision of tissues and preceding the activity of newly formed meristems, for it is only here that conversion of cells already differentiated takes place. The phenomena of wound reaction and early regeneration have recently been reviewed by Bloch, who has discussed the significance of dedifferentiation and redifferentiation for the problems here under consideration.

The other major contribution of histological regeneration to our problem has been a demonstration of the fact that when a cell alters its course of differentiation in response to a removal of part of an organ, the new course which it follows tends to be like that of a cell in normal development which occupies a similar relative position in the organ. In other words, the cell tends to follow the course of differentiation proper to the new position in which it finds itself, and thus to restore the normal histological picture just as the development of new roots and shoots restores the missing parts necessary for an entire organism. No better example of such regenerative differentiation can be found than in Vöchting's classic experimental studies on the kohlrabi tuber. He describes how the outermost layer of living pith parenchyma cells on the wound face become cutinized, develop stomata and restore a continuous and typical epidermis, a fate far removed from their normal one in an uninjured tuber. In the same way, deep-lying cells of the medulla give rise to groups of vascular elements which tend to restore the complex vascular system of the organ. Similar results

have been obtained by many other workers. Thus the continuity of strands of xylem and phloem, experimentally broken by cutting, in leaf, petiole or stem, is restored through the differentiation of a series of adjacent parenchyma cells into vascular elements which link together the severed ends of the original bundles, as Simon and Kaan-Albest have shown. In *Crassula*, *Begonia* and other plants which readily produce adventitious structures, it can be seen that normal epidermal cells entirely reverse their function and give rise to apical shoot meristems. Furthermore, in the parenchymatous tissue under such a new growing point vascular cells become differentiated which connect the new adventitious shoot with the conducting system of the organ on which it arises. These transformations sometimes merely change the character of existing cells, as when a parenchyma cell is redifferentiated into an epidermal one by the cutinization and thickening of its outer wall or into a functional xylem cell by the lignification and pitting of its wall and the loss of living contents. In other cases the change may be accompanied by the onset of meristematic activity, the old cell being finally cut up into a group of new ones which differentiate into the new type of tissue. In all cases the essential fact is the same—a cell destined for one course of differentiation meets an entirely different fate. What this shall be seems to depend primarily on its relation to the entire organ. It differentiates in a way that is proper for the position in which it occurs. More than sixty years ago, in 1878, but in terms which can hardly be improved upon to-day, this fact was stated by the great experimental plant morphologist, Hermann Vöchting, in his "Organbildung im Pflanzenreich," thus: "The particular function (character) assumed by a cell is determined primarily by the morphological position which it occupies in the living unit." Later, the same conclusion was stated for animal development by Driesch in the famous aphorism, "The fate of a cell is a function of its position." It should be of interest to botanists that this great generalization was first established from a study of plants.

What meaning has this conclusion for the student of differentiation? He will admit, at least in theory, that an excised bud or other mass of tissue has the capacity to restore the entire organism; that even a single somatic cell may retain this capacity; that profound changes in the fate of cell or tissue are produced by isolation and regeneration, and that in some way this is prevented by the membership of cell or tissue in an uninterrupted and integrated organic entity. But why, he asks, should mere *position* in this unity determine the course of cellular differentiation? No satisfactory answer can yet be given to this question. We can say only that it involves the phenomena of correlation between the diverse parts of a continuous living whole, the factors which integrate these into an organism of specific shape and size and thus, in a word, the ancient biological problem of *pattern*, which confronts us along every avenue of inquiry into the nature of organisms if we pursue it far enough. Pattern is simply the visible manifestation of organization, that attribute of living things which has so long remained an enigma for students of plants and animals.

But if differentiation is only another aspect of organization and if the course which a cell follows is determined by its place in some pattern of development, then *each* cell division must be a differential one, since the two daughter cells, even if they still appear to be alike in every other respect, are different in their position and thus in their ultimate fate. We should perhaps not speak of the mass of similar cells at a meristem as "undifferentiated," nor indeed of undifferentiated tissues at all, save in such monstrous and amorphous masses as develop when organized control is removed.

The problem facing the student of differentiation, therefore, is to learn how these differences in position are translated into differences in structure. He must do no less than explain the origin of pattern itself. Here lies the most difficult task in biology. Few pathways seem promising. Differences in the relation of the developing

structure to the external world, as between its upper and lower sides or its inner and outer portions, may establish axes and gradients, as Child has shown. These simple differences, however, can hardly account for the manifold diversity of parts with which we are confronted in organic development. With Harrison, we may look to the form of the protein molecule for the cause of pattern. We may analyze morphogenetic fields, with Weiss, or seek with Burr and his colleagues to translate them into more concrete terms of potential differences. There is yet no solution nor even a very active expectation of one. The very terms "pattern" and "field" are suspect to many as implying agencies too mystical or immaterial for orthodox biology. It is here that the advance of morphogenesis seems to have stalled, bogged down in uncertainty of fact and difficulty of conception.

But it would be wrong to paint too gloomy a picture of our knowledge of pattern and of organic differentiation. The very great progress, already mentioned, toward an analysis of the factors which affect development is very hopeful and from such studies may come the clue toward something more profound. Environmental factors and chemical and physical agencies obviously modify development and differentiation very markedly, and by their means we can greatly enlarge the organism's morphogenetic repertoire available for our investigation. It is important to remember, however, in our enthusiasm for "formative substances," hormones, vitamins, genes and similar controlling agencies, which have contributed so very greatly to our knowledge of organic development, that these are merely the messengers, the mechanism, of some more deeply seated control which knits the mass of living stuff into an organism. We are in the position to-day of one who may know much about baking brick, mixing concrete, framing timbers and making the material for plumbing, heating and lighting a house, and can modify them at pleasure, but who is entirely unable to bring these structures logically together and to build a

house out of them because he has no plan nor can he understand how one is to be drawn. We seek the architect, not the maker of builders' supplies.

But from all this uncertainty one tangible fact is emerging—the seat of the process of differentiation seems to be clear. For a generation we have heard of the importance of the nucleus, of its chromosomes, of its genes, of the various changes which go on within it. Some biologists hardly recognize that there is anything else worth considering in the cell. And yet the splendid contributions of the science of cytogenetics have yielded little as to the problem of differentiation. The nucleus certainly has its own organization, its precise spatial arrangement of genetic mechanisms, but this has no direct relation to the histological pattern. Genes certainly may modify this pattern, but they seem quite unable to establish the basis of it. The fact that every cell is genetically equivalent to every other one itself precludes the idea of a control of differentiation by the nuclear apparatus alone. Only one possibility remains—the cytoplasm. The evidence at hand from studies of development in both plants and animals indicates that this deceptively simple material, itself apparently so homogeneous and unorganized, bears within it the integrating, patternizing mechanism which parcels out to every cell its share in the organization of the whole. No nucleus alone seems able to regenerate an organism—a cytoplasmic complement is also essential. To be sure, the nucleus determines *what sort* of a pattern of differentiation shall be established, perhaps by a genic production of substances like those now so commonly employed by experimental morphologists; but the *construction* of a pattern with its precise location of specific cellular differentiations is apparently the prerogative of the cytoplasm alone. It is as though the cytoplasm contained the plan of the organism and the nucleus its specifications. One may speculate as to how this organizing and integrative capacity of living things inheres in such a fluid and seemingly unpatterned material, and how the nuclear

mechanism may have been evolved to control and direct the pattern, as does the musician his instrument or the sculptor his clay, but such speculations are of little value in our present state of ignorance. What matters to the student of differentiation is that the changes in cells and tissues, which he observes in organic development, in some obscure fashion arise in and are dependent on the cytoplasm, and that whatever chemical or physical factors are concerned in differentiation produce their effects, directly or indirectly, through this material. The cytoplasm is much less interesting and dramatic—and much more difficult—to study than the nucleus, but in it seems to lie the secret of differentiation and all kindred problems, and here we must seek their solutions.

Even such a brief review as the present one must emphasize how great and gratifying is the knowledge already gained and how considerable is still our ignorance concerning the problem of organic differentiation. We can describe and analyze, but the ultimate mechanisms are still beyond us. This should not be discouraging to the experimental histologist, however, for all the other fields of biology are equally uncertain. Organization is the basic riddle, and whoever learns its secret will teach us to understand not only why cells, tissues and organs differentiate but what a living organism really is.

EFFECTS OF GROWTH-REGULATING SUB- STANCES ON MOBILIZATION AND DEVELOPMENT

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INTRODUCTION

PROBLEMS dealing with the growth and development of plants not only involve the mechanisms whereby they enlarge and differentiate, but they must at the same time include the influence of those internal and external factors that either suppress, or allow the expression of the different potentialities for growth that are inherent within the plant. Although the influence of these factors is expressed in the size, shape and physiological functions of the plant, these outward expressions are, in a sense, superficial, since they are only the reflection of the growth patterns fundamentally laid down by the cells. Interest then lies, primarily, in the degree to which the growth potentialities are expressed by this unit—the cell.

For this approach to the problem, the environmental factors concerned may be classified rather broadly into two groups—nutritive and regulatory; and it is toward the regulatory factors that attention is here directed. Some of these are purely chemical in nature, such as growth substances, while others are non-chemical, such as temperature and light. Recently, plant scientists have begun to study intensively those that are of a chemical nature. These substances are regulatory in their effect on growth in the sense that they do not form a major part of the body of the plant. Instead, surprisingly small amounts are capable of influencing the degree to which some growth potentialities are expressed.

THE PRODUCTION OF CELLS

In considering the ways in which the growth and differentiation of cells is influenced by these regulatory fac-

tors, it seems reasonable to begin with those processes that are concerned with the production of new cells—that is, cell division. In meristematic regions, materials are most actively combined and elaborated into new cells, and the course followed by the processes concerned constitutes a physiological pattern of growth that is characteristic of these regions. The variation of regulatory factors, such as light, temperature or growth-regulating substances, does not greatly influence this series of basic physiological processes, since the final appearance or structural characteristics of the newly formed cells are essentially unchanged. These regulatory factors can, however, influence the initiation of this series of processes or, in other words, they can regulate, to some extent, the rate of cell production and the distribution of meristematic activity. For example, light can influence the distribution of meristematic growth in the stem apex of some plants (Priestly, 1926), but the structural characteristics of these meristematic cells remain unchanged. The influence of some other regulatory factors, including some organic acids, may be expressed in a more frequent occurrence of cell division in terminal meristems of roots (Hammett, 1929, 1930; Levan, 1939).

Aside from the influence of such external factors as these, there are other regulatory factors which are inherent within the plant. These are generally referred to as hormones or growth-regulating substances. They are of a chemical nature and are capable of exerting a specific influence over the rate of cell production. Some, such as traumatin, a wound hormone, may increase the rate of cell division (Bonner and English, 1937, 1938; Haberlandt, 1921; Snow, 1933), while others seem to be associated with inhibition of cell production. The inhibiting influence of growth-regulating substances on cell production is, under some conditions, exemplified by the lack of growth in lateral buds.

The controlling influence exerted by one bud on the development of another is one of the most obvious charac-

teristics of plant growth, and this behavior is profitably employed as a basis for pruning. However, the nature of this regulatory influence is, at present, a subject of dispute. No less than nine theories have already been proposed in an attempt to explain how the growth of a bud can exert an inhibitory effect on the growth of others on the same stem (Thimann, 1939).

That this inhibiting influence is, in some plants at least, a substance, is shown by the fact that it can pass from one plant to another through a protoplasmic discontinuity (Snow, 1925, 1940). Furthermore, growth-regulating substances extracted from certain parts of some higher plants, and also from fungi, brought about the inhibition of bud growth (Heyn, 1940; Mitchell and Whitehead, 1941; Thimann and Skoog, 1933, 1934). Similar effects can be seen in many different kinds of plants following the application of certain organic compounds (Hamner, 1937; Kraus and Mitchell, 1939; Thimann, 1939). It appears that this inhibiting influence is not entirely the result of the diversion of food, away from the inhibited bud toward the dominant one, since apical buds, those farthest from the supply of inorganic materials, are most often dominant. Furthermore, through the use of relatively small amounts of some synthetic growth-regulating substances, it has been possible to completely inhibit bud growth, without any noticeable redistribution of food materials (Skoog and Thimann, 1934; Snow, 1925; Thimann, 1937).

All this evidence favors the assumption that the correlative growth of buds is brought about through the production of a growth-regulating substance by the dominant bud. But the mechanism by which such a substance might inhibit bud growth is not clear. In attempting to solve the problem, investigators have been confused by the fact that substances which are capable of bringing about cell enlargement are, at the same time, influential in inhibiting the growth of buds. However, the problem has not been approached from the standpoint of gaining an understanding of the relation of these regulatory factors to the

various phases of cellular growth in a bud—that is, cell production, cell enlargement and vacuolation, and cell differentiation. Nor is there reason to believe that a complete understanding will be gained, until such an approach is made, since these phases of cellular growth are fundamentally concerned in experiments that deal with bud inhibition.

Thus, those processes that are concerned with the production of new cells in the meristems of plants are influenced by external factors and by factors inherent within the plant. But the potentiality for division is not confined entirely to the embryonic cells of meristems, or the cambium; instead, it is retained by almost all vacuolated cells during maturation (Bloch, 1941), and its expression may be induced in parenchymatous cells of most organs. In considering, then, the production of new cells in a plant, this potentiality must be taken into account. Its expression can be brought about by a number of external factors, some of which are mechanical, or physical, such as wounding, humidity or even the orienting of a stem from a vertical to a horizontal position. Recently it has been found that there are also a number of chemical substances that are capable of inducing some types of vacuolated maturing cells to divide.

A large amount of effort has been directed, within the last few years, toward gaining a better understanding of these chemical substances, as related to cell growth. This research has been, in a sense, divided. On the one hand, a number of investigators have been interested mainly in these substances as they affect the production of new cells, their differentiation and their organization into tissues, tissue-systems and organs. Such responses have been induced through the application of relatively large amounts of these synthetic regulatory substances. In contrast, other investigators have concerned themselves mainly with a study of the influence of these substances on dimensional changes of the cell. In these experiments relatively small amounts of growth-regulating substances

have been applied. Of interest is the fact that a large amount of effort on both sides has been directed toward an understanding of changes induced in maturing cells, and to a lesser extent in cambial cells, while little or no attention has been directed toward the effect of these substances on the more embryonic, undifferentiated cells of primary meristems.

Considering cell division, as induced by growth-regulating substances, an outstanding characteristic is that some cell types are specific in their ability to respond to different substances. As an illustration, mitosis can be induced in the parenchymatous cells of most stem tissues through the application of a number of compounds, such as indoleacetic, indolebutyric and naphthaleneacetic acids. Following division the derivative cells differentiate, and often become organized into roots or, in a few instances, into stems. In contrast, the application of tryptophane, which also induces the parenchymatous cells of some stem tissues to divide, does not affect pith cells, nor are roots ultimately produced in the treated portion of the stem (Kraus, 1941). Naphthalene acetamide, on the other hand, brings about an accelerated rate of cambial activity, and new cells thus produced differentiate as secondary xylem and phloem (Kraus and Mitchell, 1939). Indoleacetic, phenylacetic and naphthaleneacetic acids can also accelerate cambial activity, but to a lesser extent, and the response is not accompanied with the formation of an appreciable amount of secondary thickening. Some natural growth-regulating substances have been obtained from pollen, and these are also capable of inducing parenchymatous cells of specific tissues to divide (Mitchell and Whitehead, 1941).

Thus the potentiality for division is often accelerated in the cambium, or expressed by maturing, vacuolated cells, when their course of differentiation is changed, through the application of relatively large amounts of either natural or synthetic growth-regulating substances.

MOBILIZATION

Back of these more or less obvious histological or morphological expressions of cell growth, lie physiological changes that are induced through the influence of these chemical regulators, changes that bring these relatively mature, vacuolated cells back to a more embryonic state. In these substances, then, there is at our command a tool which can be used to initiate, in certain cells, a series of physiological processes which lead to the production of new cells, and ultimately to the development of new tissues and organs. At present, little advance has been made toward an understanding of this phenomenon. In our attempts, however, it has been observed that an increase in cell volume is usually the first outward evidence that this series of processes has been initiated. But the exact nature of the primary factors concerned, or the mechanism whereby this series of processes is initiated, is not understood. Nor is there a clear understanding of the many subsequent physiological processes which lead to mitosis, and ultimately to the differentiation and organization of tissues and organs.

It might be of interest, however, to enumerate here some observations concerning the food materials required for these processes, and to correlate the transport and utilization of these with successive changes as they occur following treatment. Thus from the structural viewpoint, the initial change is most often an increase in cell volume, followed by an increase in the density of cytoplasm, cell division and finally the differentiation of the resulting new cells. From the standpoint of cellular nutrition in connection with these responses, the first apparent change is that reserve carbohydrates in the form of starch are made available through hydrolysis. Sugars and inorganic substances may be transported from other parts of the plant (Borthwick, Hamner and Parker, 1937; Kraus, Brown and Hamner, 1936; Snow, 1933; Stuart, 1938; Unpublished Data). These materials are rapidly utilized in the synthesis of protoplasm, as indicated by

the remarkable rate of increase in the density of the cytoplasm which precedes mitosis. Following the division of the original cells, mitoses often occur in rapid succession, and a large amount of inorganic and organic material is used in the production of new cells, and eventually in their maturation and differentiation (Mitchell and Martin, 1937; Mitchell, 1940). This meristematic activity sometimes becomes so intense as a result of treatment, and the utilization of food so great, that relatively large amounts of these materials are mobilized from other parts of the plant to the treated region (Stuart, 1938). It appears that, under these conditions, the inhibition of terminal bud growth, which is associated with the application of relatively high concentrations of growth substances to stems, may in part result from the mobilization of food materials away from the bud.

CELL ENLARGEMENT

The expression of a potential growth pattern by an organism is not only influenced by variations in those processes that concern the production of cells, but also by variations in subsequent phases of cellular growth, including cell enlargement, and differentiation.

It has long been recognized that the potentiality for cell enlargement is most strikingly expressed in the elongating regions of stems and roots or in expanding leaves. This expression is exemplified in tropic responses, which are induced by such external factors as gravity and light. Within recent years a somewhat better understanding has been gained as to the mechanism of this phase of cellular growth. The discovery that certain synthetically prepared chemical substances are capable of bringing about growth by cell enlargement opened a fertile field for investigation concerning this problem (Kögl, Haagen Smit and Erxleben, 1934). It was found that, within limits, a quantitative relationship existed between the amount of these substances applied and the degree to which cell enlargement was expressed. Although the sensitivity of cells

varies with the different phases of their growth and differentiation, a quantitative biological test for natural or synthetic substances capable of inducing cell enlargement was devised (Went and Thimann, 1937). Methods for the extraction of natural growth-regulating substances are crude, and the test non-specific; nevertheless, it has been possible to demonstrate that certain regions of a plant, such as growing buds, often yield a relatively large amount of those substances capable of inducing cell enlargement.

In some specific instances the production of these substances in one part of a plant can be correlated with the expression of growth by cell enlargement in other parts of the plant (Boysen Jensen, 1936). This concept, however, does not always hold, as is illustrated by the fact that the presence of growth-regulating substances in rapidly growing terminal buds is sometimes associated with the lack of growth of lateral shoots. Nevertheless, more and more evidence is being accumulated with support of the conclusion that growth by cell enlargement is, in some cases, inherently associated with an internal growth factor of a chemical nature. Tropisms are considered by some to be the expressions of growth by cell enlargement brought about by the unequal distribution of these internal regulators, their redistribution being conditioned by external factors such as light, and the effect of gravity.

Thus a somewhat clearer understanding of the role of these regulatory substances in cell enlargement has been gained within recent years. Considerable study has been directed toward the mechanism by which these substances induce cellular growth. Although the first observable effect following treatment is an accelerated rate of protoplasmic streaming, it is questionable whether this response is directly concerned with the enlargement of the cell (Sweeney and Thimann, 1937; Went, 1939). Of basic importance are changes that occur in the cell wall which lead to an increase in its area. The nature of these wall changes are considered by some to be a decrease in the

resistance offered by the wall to internal pressure, followed by an increase in volume of the cell. Such an increase in size is, in the end, non-reversible, because of the incorporation of new wall substance. Others hold the view that through the influence of a growth substance, a series of processes are initiated in the cell, which lead directly to the synthesis of new wall substance, and its deposition through intussusception, resulting in an increase in wall area (Boysen Jensen, 1936; Heyn, 1940).

The validity of either of these theories can not be proven without extended study, and it is clear that neither one deals directly with the primary cause of growth by cell enlargement. Yet they make clear beyond a doubt the fact that no explanation of cell enlargement would be complete without a consideration of the effect of chemical activators.

DIFFERENTIATION

There is left, then, a consideration of the final phase of cell growth—the differentiation of cell parts. Following the meristematic phase of growth a new cell enters upon a course of differentiation, becomes diversified in structure, and ultimately represents a cell type, specialized as to function with respect to the organism as a whole. From the present viewpoint, this series of developmental changes can be recognized as a primary pattern of differentiation. Primary, since a secondary pattern is sometimes expressed by the derivatives of those cells that retain their potentiality to revert to the embryonic stage. That is, when a parenchymatous cell is induced to revert to a more embryonic condition and divide, then some of the derivatives may enter upon a second course of differentiation, a new pattern of development, that results in a new cell type.

It seems evident that once a cell has started to develop, the degree to which differentiation is carried may vary, but the course of development does not change, except indirectly through reversion to the embryonic state. The potentiality to revert is sometimes induced in parenchy-

matous cells by such factors as humidity (as illustrated by the rooting of cuttings kept under moist conditions), wounding, and by the application of different synthetic growth-regulating substances (Bloch, 1941; Dorn, 1938; Kraus, Brown and Hamner, 1936).

But from the standpoint of the organism as a whole the course of differentiation that is expressed by a single cell is of less moment, since a maturing cell ultimately takes its place with others to function only as a part of the whole. Finally then, the processes we have considered—cell production, enlargement and differentiation, with all their variations—are correlated in such a way as ultimately to express the intricate growth pattern of the organism, a pattern that is the product of inherent genetic potentialities, operating in an ever-changing environment.

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DISTRIBUTION AND ORIGIN OF SOME EASTERN OCEANIC INSECTS¹

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THE foundations of our knowledge of biogeographical distribution are rooted in taxonomy, but any comprehensive studies must also impinge upon geology, paleontology, genetics and evolution. To interpret correctly the Pacific faunas and floras, the geological, paleontological and biological histories of other insular areas and continents must be taken into account. Thus, in a study of the origin and distribution of Pacific insects, it is necessary to consider all groups of organisms found in the endemic insular faunas.

Much confusion exists in literature and in the minds of students concerning Oceania and its inhabitants. Many authors feel that the Pacific is shrouded in mystery, and they begin their studies biased and under false impressions.

I. GEOLOGICAL FOUNDATIONS

Though much of the geological research in the Pacific basin is in a reconnaissance stage, the major structural and physiographic features have been outlined, and for some of the islands detailed studies have been completed. In general, it may be said that in so far as they are related to biological problems, the fundamental geological factors, topography, climate, soil, the indigenous floras and faunas and proven human immigrations are fairly well known. It may be assumed that most geologists know

¹ Excerpts from an address delivered to The Pacific Science Survey Group of the Hawaiian Academy of Science, University of Hawaii, Honolulu, March 21, 1940. Unless otherwise stated, the opinions expressed in this paper are principally those based upon the study of insects. My sincere thanks are due to several workers who have offered constructive criticism and especially to Dr. H. E. Gregory for his aid in editing the original manuscript.

more about geology than do most biologists, and therefore much of the controversy between biologists and geologists in regard to the extent, distribution and age of lands and seas could be avoided if the biologists would be more careful in their assumptions and give more credit to the work of geologists.

As the result of geological study, it is known that the Pacific basin is underlain by heavier rocks than are the continents bordering it; that in the continents lighter rocks rest on heavier under layers; and that continental shelves extend for various distances under shallow coastal waters and then terminate abruptly at the edge of the deep water which is remarkably uniform at 12,000 to 14,000 feet deep in the true Pacific basin; that the volcanoes now above sea level have arisen from great depths and have brought with them heavy basalts and no light rock, and that in no islands in the eastern Pacific basin have true continental rocks been found. So far as it is known to geologists, the only pre-existing extensive land masses in the tropical Pacific since the rise of modern floras and faunas are those west and north of Australia and on the New Guinea-New Caledonia-New Zealand axis, possibly extending eastward to near the Tongan trough in the neighborhood of Fiji. There is no geological evidence to indicate the existence of any large land masses east of Tonga.

However, in spite of the geological evidence, some biologists accept the dictum of Meyrick,² who writes, "A rise of 12,000 feet in the sea-bottom of the South Pacific is required to show these results, but I entertain no doubt that such an elevation must have existed since the Eocene period, because it is absolutely the only explanation possible" to account for the distribution of a genus of moths represented by species in Australia and southeastern Polynesia. Meyrick nonchalantly raised over 2,000,000 square miles of land to account for the distribution of a few moths and sank it into oblivion, leaving no trace of

² *Trans. Ent. Soc. London*, 24: 271, 1926.

the major diastrophic action necessary to raise and lower such a block of sea-bottom.

So many continents and land bridges have been built in and across the Pacific by biologists that, were they all plotted on a map, there would be little space left for water. Whenever a particularly puzzling problem arises, the simplest thing seems to be to build a continent or bridge, rather than to admit defeat at the hands of nature, or to consider the data at hand inadequate for solving the problem. Most of the land bridges suggested to account for the distribution of certain plants and animals in the Pacific create more problems than they solve. If the central³ and eastern Pacific ever included large land areas and land bridges, there should be some indication of the consequent peculiar development of the faunas and floras, but there is no such evidence.

As I see the problem, the eastern oceanic insect faunas could not have become what they are with any great land areas or bridges in the central Pacific; their very character precludes those possibilities. The known groups are the results of overseas sifting; there are too many groups lacking for any other means of dispersal to have been involved. If biologists want to find out the ages of their insular floras and faunas, let the geologists tell them the probable ages of the lands upon which they exist; then, and then only, can they begin to compute the ages of locally developed groups with some degree of accuracy.

Geologists argue among themselves as to how atolls were formed, but apparently few of them advance the thesis that they may be formed by several different methods; some may be caused by subsidence, some by elevation, others by glacial control, others by marine planation. Geologists, like biologists, often fail to take into consideration the inherent variability of nature and

³ For our purposes, it is convenient to consider those islands east of the International Date Line to Easter Island as being in the central Pacific, but they belong to *eastern Oceania*, because Oceania is a term applied to the islands of "Polynesia, Micronesia, Melanesia, Australasia and Malaysia" (Century Dictionary).

search for one theory of explanation of natural phenomena. It is probable that much light could be thrown upon the atoll problem by a comprehensive study of the underlying framework of the atolls of the Tuamotu archipelago; some of those islands are large enough to enclose the entire islands of Tahiti and Moorea with room to spare. Present knowledge of the great atolls of eastern Polynesia rests largely on conclusions reached by geologists who have never seen them.

Though the evidence for former extensive land masses in the Pacific basin is lacking, it is probable that high islands other than those represented on maps existed in past ages. There are numbers of volcanic cones below sea level, some of which come close to the surface.⁴ There might well have been some high islands between Hawaii and the south and western Pacific. Such islands, when above water, may have been used by plants and animals as stepping stones. There have been shifts of sea level of at least 1,000 feet in Hawaii; such shifts in the south Pacific would change the appearance of the map. Some fast constructions seem necessary to account for such steep-sided, lofty masses as isolated Wake Island, which arise abruptly from great depths, and when it is recalled that the Mexican volcano, Jorollo, burst through a level plain and rose 1,600 feet in a single night, it is easy to think of similar occurrences and great changes in the central Pacific. Such phenomena are not, however, inconsistent with the belief in a relatively stable sea bottom. At least for eastern Oceania the distribution of insects could have been accomplished with little change in the present proportions of land and sea.

II. DISPERSAL

It might be said that the reliable knowledge of the distribution of oceanic insects consists of the facts that they

⁴ For example, submarine mountains rise as much as 13,500 feet about 140 miles S.S.W. of Honolulu; Raita Bank has a shoal area about 25 miles long, and in some places it has only about 50 feet of water over it; there is a submarine range of high mountains about 200 miles southwest of Necker Island.

occur on various islands and have been derived from certain related faunas of other islands or continental areas. It is not known how the ancestors of each group of insects reached their present habitats. However, to me the crux of the problem is not so much how the various groups have been spread, but from what areas they have been derived and to what areas they have been distributed. Insects do inhabit all habitable insular groups no matter how great the isolation, and, therefore, they possess some advantages that better fit them for selection by agents of overseas distribution than do some other animals. It seems appropriate to speak of a Polynesian flora or a Polynesian fauna, not with the understanding that this fauna or flora has originated in Polynesia and is thus characteristic of it alone, but rather that the inhabitants of the area are the descendants of those which the selective agents of distribution have dispersed. This statement does not preclude the possibility of later local developments within the area, however. Many of the insect genera inhabiting the central Pacific are flightless, and because they inhabit islands separated by hundreds of miles of open sea, their distribution is obviously independent of their ability to walk. These insects have crossed, and undoubtedly still are crossing, large bodies of sea, not because they have wanderlust, but because of forces beyond their control—forces undoubtedly adverse to their general well-being. It is in the methods of transport that so many students disagree and so many hypothetical data are advanced. In my opinion, the ways and means of dispersal is a field unsuited for dogmatic statements. It is highly improbable that dispersal can be assigned to any one factor; it is the result of a combination of factors acting over long periods of time, and different groups of organisms may be dispersed by diverse methods. Chance and time accomplish much, or, as Herodotus put it in 400 B.C., "If one is sufficiently lavish with time, everything possible happens."

The three principal means of transport generally

advanced are: marine drift, wind and aid from other organisms.

Marine drift. Thousands of bottles thrown overboard from vessels sailing the Pacific have given a pretty good idea of the set of normal contemporary currents. Logs from northwest America are washed up on Hawaiian shores, and fish-net floats from Japanese waters are brought to these islands by the currents. Bottles thrown into the sea off the Central American coast or south of Hawaii drift to the Philippines or to islands between the Solomons and Borneo, and some of these bottles make a speedy journey. These are the normal currents of to-day. In general, however, the endemic insect faunas of the south Pacific lack American elements, and, therefore, these normal currents can not be looked upon as the agents responsible for their distribution. Probably the distance and time factors involved eliminate the possibility of the introduction of an American element into the south Pacific by normal drift; but what about abnormal currents that flow opposite to the normal currents? Currents set up by cyclonic disturbances and anti-trade winds are quite different from normal drift.

There are numerous records of live animals being transported for considerable distances by sea drift. Large rafts of debris have been seen floating out to sea from the rivers of Borneo, Fiji and other islands. I have seen large trees with branches riding high out of the water floating out to sea after a Tahitian storm. A Solomon island crocodile came ashore in Fiji and killed several natives before it was destroyed. I found a live ant on a floating leaf in the Korokoro sea several miles from land. Wheeler⁵ described a new species of ant from a colony that was carried from Brazil to San Sebastian Island in a floating log. A fur seal came ashore on the tropical island of Mangaia in the Cook group. Dr. C. H. Edmondson recently gave me a well-developed, living larva of a longhorn beetle, which he took from a dead

⁵ *Psyche*, 23: 180, 1916.

mangrove tree found afloat in Hawaiian waters. A living boa constrictor floated about 200 miles to St. Vincent Island on a tree. This list, which could be greatly enlarged, is indicative of possibilities.⁶

I believe it probable that part of the insect faunas of the central Pacific islands may have been derived from ancestors that drifted with marine currents. The larger part of the faunas of high oceanic islands⁷ are made up of weevils, especially cossonine weevils, which are quite resistant to wetting. I do not believe, however, that this method of transport accounts for the dispersal of the bulk of the genera. It seems improbable that certain land snails, for example, could be so transported. The great difficulty in the dispersal of animals from one locality to another on floating vegetation appears to be not so much dependent upon their being cast adrift and being transported, but rather in their landing in another locality and becoming established in a foreign and perhaps hostile environment. Sea beaches upon which flotsam is cast are not usually favorable environments for the establishment of most terrestrial organisms.

Winds. Normal winds are usually not strong enough to transport even small insects for significant distances across open bodies of sea. The trade winds of the south Pacific blow from the east, away from the American continent. Hurricanes, however, have often swept from the west, crashed through insular forests, stripping trees of their leaves and twigs, churned across the sea, and passed over the islands eastwardly of their origins. It is these abnormal winds which I believe have accounted for the distribution of the bulk of the insects of the south central Pacific.

There are numerous records of the transport of insects and other animals by wind. Hundreds of moths were taken at sea almost 1,000 miles from their homes in the

⁶ Additional notes in summary form may be found in Hesse, Alce and Schmidt, "Ecological Animal Geography," 1937.

⁷ Oceanic islands are either coralline or volcanic; continental islands contain metamorphic or light rocks.

Cape Verde islands. Large numbers of dragon flies were driven by strong winds 700 miles from Sumatra and Java to Cocos-Keeling island. De la Torre⁸ has shown that certain Cuban land snails have been carried to Florida by hurricanes; he goes so far as to say that he can plot out colonies in the exact paths of known hurricanes.⁹ Mosquitoes and other insects are regularly carried almost 100 miles by wind from Cuba to Rebecca Shoal light. In 1830 Kotzebue recorded swarms of butterflies and land birds about his ship during a heavy wind while he was between the Ellice and Gilbert islands. Land birds are frequently blown far by storms and appear in unexpected places. A Chinese cuckoo was recently blown to Wake island by a westerly storm of several days' duration. A pair of North American kingfishers, after probably being blown out to sea, arrived by flight in Hawaii and were observed fishing for some time before they disappeared. In Hawaii, winds blow wingless mealybugs about from one pineapple field to another.

The extensive studies recently made by P. A. Glick¹⁰ of the distribution of arthropods in the air provide much pertinent information. Glick's work covered a period of five years with over 1,000 hours of airplane trap flights made. Glick concludes that "some of the most dangerous insect enemies of cultivated plants and the carriers of dreaded diseases of man and animals spread to distant places with the aid of air currents." Not only were thousands of insects taken at various altitudes up to 14,000 feet, but spiders and mites, wingless creatures, were not uncommonly captured. One spider was taken at 15,000 feet. Some opponents of dispersal by wind say that certain insects are weak fliers and are therefore not capable of traveling great distances on the wind. These weak fliers are just the insects which Glick found to be

⁸ See note by Thone, *Science*, 86: 2232, suppl. p. 8, 1937.

⁹ W. J. Clench discusses hurricane distribution of land snails in the Bahamas in *Bull. Mus. Comp. Zool.*, Harvard, 80 (14): 489, 1938.

¹⁰ U. S. D. A. *Tech. Bull.* 673, 1939.

the most abundant in the air and which were carried to the greatest heights. Heavy-bodied, strong-flying insects were not taken high in the air. Not only were winged adult insects collected, but larvae, nymphs and wingless adult insects were captured as high as 14,000 feet. Glick says these wingless forms are all at the complete mercy of the upper air currents. F. C. Fraser,¹¹ speaking of the widespread damselfly, *Ischnura aurora* Brauer, which is found from western India to southeastern Polynesia, says, "In Coorg I have often watched this species taking flight after emerging, and have followed it with my eyes as it rose almost perpendicularly in the air, until finally lost to sight at a great height. Such a frail, tiny insect floats like a piece of gossamer, and is borne by the upper air currents to immense distances. In fact its very weakness becomes a source of strength, enabling it to spread over, and populate a vast area." Glick found that convection currents were responsible for carrying large numbers of insects high in the air, and that, during periods of strong winds, more specimens were captured than during calm periods. It appears that convection currents may carry insects into the air, even as high as the anti-trade-wind zone. Upon attaining such high elevations considerable distances could be traversed. Winds with high velocities are not uncommon over the Pacific. It may be noted that the endemic Hawaiian spider fauna is made up only of those groups that can be wind-borne; all other groups are wanting.

Dispersal aided by other organisms. Aid in the dispersal of plants and animals by other organisms, principally birds and mammals, has received much attention, and many factual data have been accumulated.¹² There is no reason to doubt such dispersal in continental areas, but there appears to be considerable reluctance to recog-

¹¹ *Insects of Samoa*, 7 (1): 23, 1927.

¹² Extensive discussions bearing on this subject may be found in many works, including H. N. Ridley's "The Dispersal of Plants throughout the World," 1930; H. B. Guppy's "Plants, Seeds and Currents in the West Indies and Azores," 1917, and Darwin's "Origin of Species," chapter 12.

nize the possibility that dispersal aids in overseas populating of islands. However, such objections are untenable in the face of abundant data.

Pseudoscorpions regularly attach themselves to insects and are carried far and wide in this manner. I took a live bark beetle from deep among the feathers of an owl in the highlands of Fiji. The owl is a wide-ranging creature. Perkins records finding a living land snail attached to a Hawaiian bird. A mallard duck shot in the Sahara had snail eggs on its feet. Fresh-water snail eggs have been found on strong-flying water beetles, and many small animals are transported from one body of water to another by insects. Some sea birds nest in mountain forests among native vegetation and might be expected to serve as agents of dispersal. Shearwaters and tropic birds nest as high as 4,000 feet or more in the forests of some islands. In the interior rain forests of Samoa, I have observed white-tailed tropic birds alighting time after time on the branches of large trees covered with epiphytes and have seen them nesting in the crotches of the trees and in *Asplenium nidus* ferns. As graceful as these birds are in the air, they are most clumsy when on trees. When they alight, they beat the surrounding foliage with their wings and claw it with their feet. These habits appear to fit them admirably for having insects, land snails and seeds lodge on their bodies and thence be carried to new localities by the wide-ranging birds. Some sea birds burrow in soil or nest on the ground, and it appears to me quite possible that insects, seeds and even land snails may become attached to them. There is a well-known Boreal element in the endemic Hawaiian flora and, to a lesser degree, in the Hawaiian fauna. I wonder what the golden plover or migratory ducks bring with them on their annual visits from the north, and what they bring back with them after their sojourns in the islands to the south. According to E. H. Bryan, Jr. (personal communication) more than 40 species of birds that are either strays or migrants from

North America are seen commonly or rarely in Hawaii. Of these, five in the plover and sandpiper families and two or three ducks are regular migrants. Plovers have been known to fly about 3,000 miles in ten days. It appears to me that the rôle played by birds in the dispersal of the terrestrial floras and faunas of eastern Oceania may be greater than is recognized at present.

One of the most conspicuous features of the insect faunas of the eastern oceanic islands is the entire absence of some large groups, families and orders common to all continents. They have been eliminated by the selective agents of overseas dispersal. Scarab beetles comprise one of the most dominant groups of all the continents, yet there is not a single native species on the central Pacific islands. Most of them are subterranean in their larval stages, and most are strong fliers. The family is greatly developed in western Oceania. To my knowledge, the only native beetles that have true subterranean larvae that occur on the islands of Oceania east of Samoa belong to the flightless genus *Rhyncogonus*—but the eggs of these weevils are deposited on leaves. The absence of endemic Chrysomelidae, or leaf beetles, from Oceania east of Samoa is difficult to explain; they are extensively developed in the western Pacific. With few exceptions, the entire endemic beetle fauna of southeastern Polynesia is composed of small, predominantly flightless species that breed in dead twigs, dead leaves or in or under dead bark, and these forms are more extensively developed than any of the other groups of terrestrial animals in that region.

We will do well to keep in mind Darwin's remark, "How ignorant we are with respect to the many curious means of occasional transport."

III. DISTRIBUTION

The distribution of oceanic insects is not so simple and orderly as it may first appear. It is complex. There are many anomalies, many internal and local developments,

many puzzling problems. However, careful study of enough groups will result in obtaining an average distribution that does show a definite pattern.

In the belief that the desired information can be more satisfactorily conveyed to the reader by diagram than by an expanded discussion in this section, I present here a few charts from a series I have prepared to show some typical distributions of oceanic insects.

IV. ORIGINS OF INSULAR SPECIES

Although much has been written about insular speciation, little is known concerning the actual mechanisms at work or the speed of evolution among insular faunas. I have read remarks to the effect that no factors not found on continental areas exist on islands to account for any more rapid speciation. I believe that such statements need close scrutiny. One must see islands and actually work among their floras and faunas to appreciate the special conditions surrounding their populations. But even given the opportunities, some biologists are misled and arrive at false conclusions. It appears difficult for students unfamiliar with oceanic islands to understand the diversity of ecological situations that may exist in a small area.¹⁴ Different sides of a ridge may present very different environmental conditions. Within a distance of two to five miles one may pass from a low-lying, hot, dry region, in which xerophytic vegetation thrives, to a cool rain forest rising several thousands of feet into the wet, windswept cloud zone. Such diversity in habitat must exert a great influence on the development of the biota and may have been a major contributing factor to the extensive speciation found in the tropics. Geneticists have not yet studied indigenous oceanic populations in detail in the field.

One of the characteristics of the insect faunas of the

¹⁴ For those interested in additional information, reference can be made to the First Progress Report of the Territorial Planning Board, published in Honolulu in 1939. Plates 8, 16-23, 50-56 are maps that give data on rainfall, soils, climate, etc.

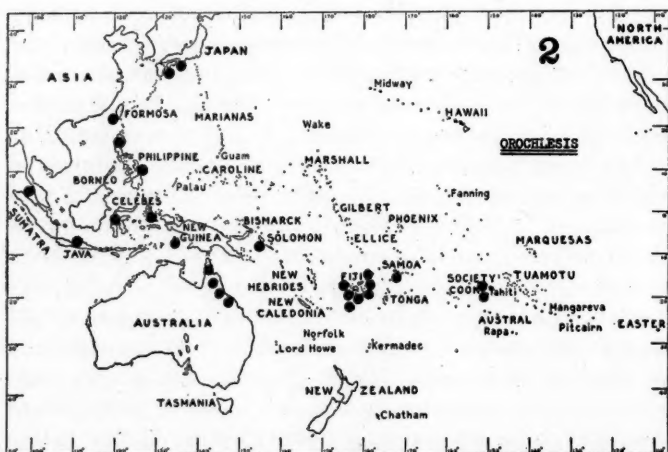
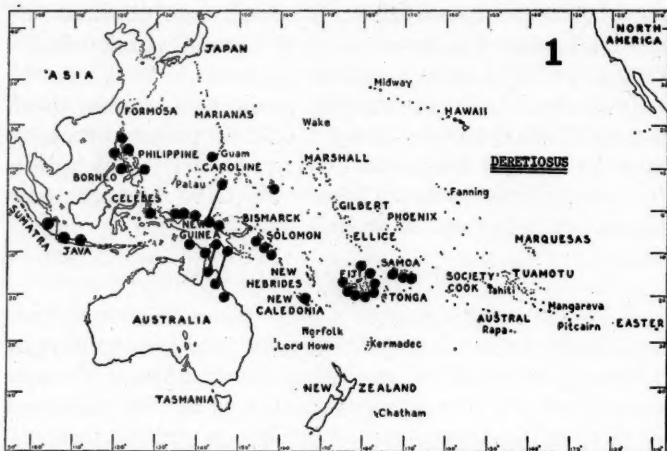


FIG. 1. Known distribution of the species of *Deretiosus* (Coleoptera, Curculionidae). Each dot indicates a species; those dots connected by lines indicate that the same species is found in two localities separated by sea. In this and the other charts, the dots show only the approximate position of the species or refer to the island group in which they occur, because the small size of the map does not permit the exact localization of each species. This chart is typical of the distribution of many Indo-Pacific insect genera, charts of whose distribution could be superimposed upon this chart with but minor

normal¹⁵ high islands of eastern Oceania is that they harbor comparatively few genera that have a disproportionately great number of species within many of those genera. For example, the weevil subfamily Cossoninae averages about 2.5 species per genus in America north of Mexico, but in Hawaii there are more than 8 species per genus. Casey gave the average of North American species of Cerambycidae at 3.0 to the genus; there are more than 10 to the genus in Hawaii. One genus represents the coleopterous family Aglycyderidae (Proterhinidae) in Hawaii, but that genus contains 175 described species. *Hypòsmocoma*, a genus of small moths, contains about 220 described species in Hawaii. There are more than 170 described species of the hymenopterous genus *Sierola* in Hawaii. There are more species of *Drosophila* in Hawaii than in all North America. Hawaii has an area of only about 1/1200 the size of North America but, according to Dr. C. Montague Cooke, Jr., it is probable that there are about as many land snails in Hawaii as there are in North America. I should emphasize the fact that such explosive speciation is not confined to insects and land snails, but also call attention to the similar development of genera, species and subspecies among Hawaiian drepanid birds, the birds of the Galapagos, and among plants. Among the islands of the Indo- and Austro-Malayan subregions there is said to be about 250 kinds of *Rattus* as against 3 for North America. On the other hand, there are genera which have not developed species complexes in the islands. *Parandra*, for example, is considered one of the most primitive genera of the Cerambycidae (longhorn beetles) and is world-wide in distribution. It has only one endemic species in Hawaii and only one in Fiji. I see no reason for considering that

¹⁵ By normal I mean the high forested islands in contrast to atolls, newly emerged volcanic cones and low, barren or semi-barren remnants of islands.

differences in extent of distribution. The species of *Deretiousus* are fully winged and capable of active flight; the larvae live in dead wood.

FIG. 2. Known distribution of the species of *Orochlesis* (Coleoptera, Curculionidae). All the known species are active fliers; their larvae are found in dead wood.

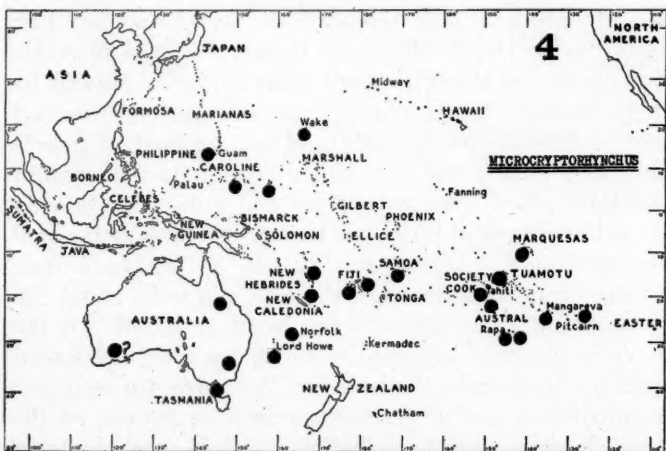


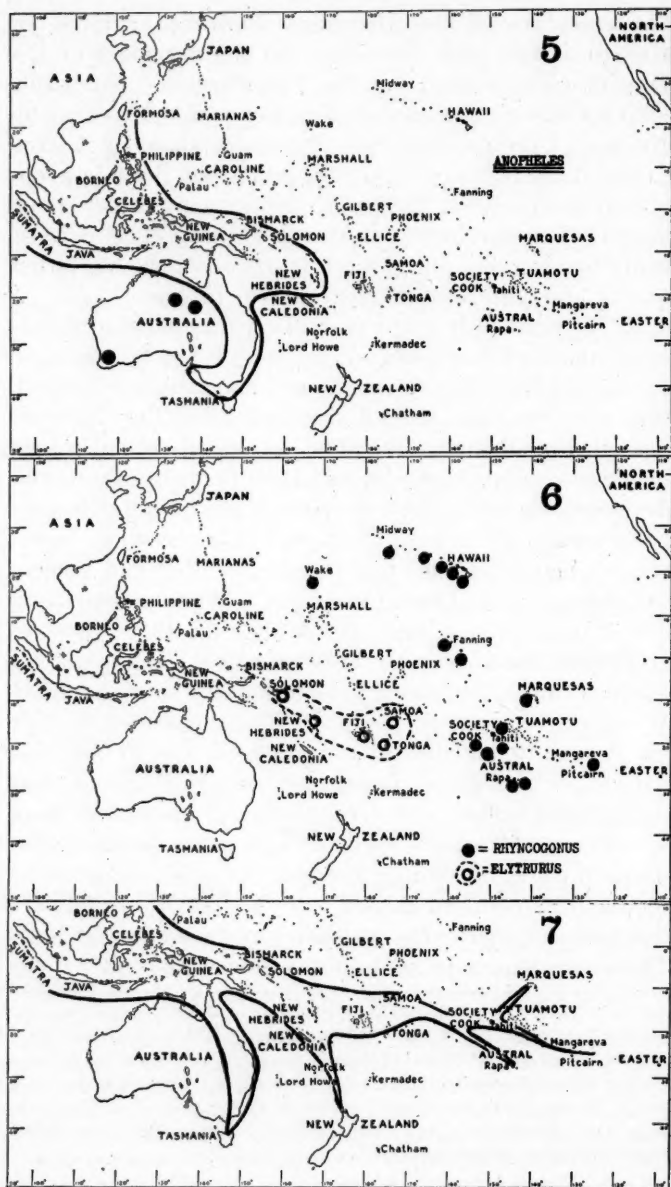
FIG. 3. Known distribution of the species of *Trigonopterus* (Coleoptera, Curculionidae). All the species are flightless; I believe that the larvae are dead wood feeders.

FIG. 4. Known distribution of more than 150 species (some undescribed) of *Microcryptorhynchus* (Coleoptera, Curculionidae). Most of these tiny beetles are less than 2 mm long and all of them are flightless; their larvae live in dead twigs, limbs and fern fronds. Each dot on the chart indicates the presence of the genus in a given locality, but does not indicate the number of species present in each region. For example, there are 43 described

the ancestors of the Hawaiian *Parandra* arrived in Hawaii at any later date than did the ancestors of the *Plagithmysus* group; yet the *Plagithmysus* group has split up into six groups called genera and no less than 95 species. I do not know why *Parandra* should not have followed the course of *Plagithmysus*—but there is probably a good reason, hidden at the moment, which some geneticist might interpret in the future. The rate of evolution is certainly subject to considerable variation among and within different groups of insects.

In the ages past great floras and faunas were developed, and they flourished and waned. When new groups, such as the Sequoias, dinosaurs and marsupials appeared, they developed and spread vigorously, but they have all dwindled or disappeared. The marsupials found a safe haven in insular Australia and have persisted there and developed up to the present time. Pacific insular faunas have essentially a similar story. When certain vertebrates originated, they had little opposition and became widespread and diverse in form. When competition arose, their ranges were cut down until they were restricted to certain areas or became extinct. In the central Pacific islands, the insects have followed a similar course. When a given species arrived that was destined to split up, it found quantities of unused food, few or no competitors, a variety of unoccupied ecological niches, and it expanded rapidly and divergently until a typical large species complex was formed. If a parasite arrived later, its path could be similar, because the same situation of food and competition existed, and the parasite could take the same explosive course that was taken by its host. These complexes prevail in the lower as well as the

species in Rapa. Knowledge of the distribution of this genus has expanded greatly in the last few years. If, for example, Bishop Museum's Mangarevan Expedition of 1934 had been sent out ten years earlier to Rapa alone, a very different interpretation of facts might have been made by an unwary worker, for it could have been shown that there were 43 species on Rapa, three in Australia and none elsewhere. Some writers have based broad conclusions on such incomplete data.



higher orders, from *Leptogryllus* in the Orthoptera to *Sierola* in the Hymenoptera, from *Megalagrion* in the Odonata to *Drosophila* in the Diptera. Many of the large species complexes of Hawaii can well be the descendants of a single fertilized female immigrant. Research has led me to believe that new lands opened for colonization are conducive to speciation. To-day competition is coming in; ranges are being more and more limited; certain species, genera and families are being driven out of some areas, reduced in numbers or are becoming extinct. Many endemic Hawaiian moths taken by Mr. O. H. Swezey 25 to 30 years ago are now rarities and are heavily parasitized by introduced parasites. Almost the entire endemic beetle fauna of the Hawaiian lowlands has been exterminated throughout the range of the voracious introduced ant *Pheidole megacephala*.

Rate of Speciation. Those who assign great ages to the central Pacific islands cite numerous examples of apparently slow evolution. Some groups of insects have gone through the Tertiary or longer with little change. Wheeler¹⁶ has shown that ants were about as fully developed in the Oligocene (as shown by Baltic amber fossils) as they are to-day. They had their casts, their hosts, their parasites, and even mites were carried on them in the same places as to-day. Many modern genera of ants now foraging fields and forests had their representatives

¹⁶ "Social Life Among the Insects," p. 6, 1923.

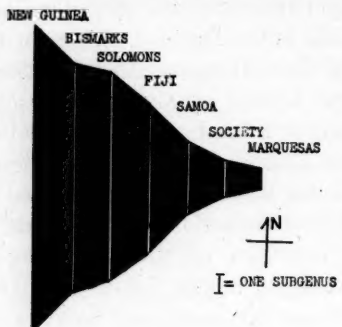
FIG. 5. Known distribution of *Anopheles* mosquitoes and malaria in the Pacific. Some discrepancies may exist in the continental Australian part of this chart because of my lack of detailed information from that region. It is interesting to note that *Anopheles* occurs in islands only a few miles away from New Caledonia, but that they are not known in New Caledonia.

FIG. 6. Examples of peculiar localized or internal developments, showing the distributions of the species of the genera *Rhyncogonus* and *Elytrurus*. Both genera belong to the same group of genera (Curculionidae, Celeuthetini), which is widely spread in the Indo-Pacific regions. The circles and dots do not indicate individual species, but only the presence of the genus. *Rhyncogonus* contains 96 species, *Elytrurus*, 36.

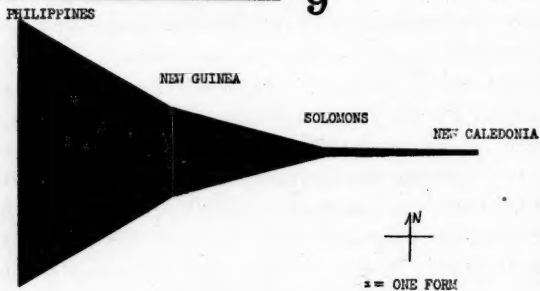
FIG. 7. A rough diagram indicating the average normal derivations of the endemic insects of the South Pacific.

SUBGENERIC DISTRIBUTION OF MOSQUITOES

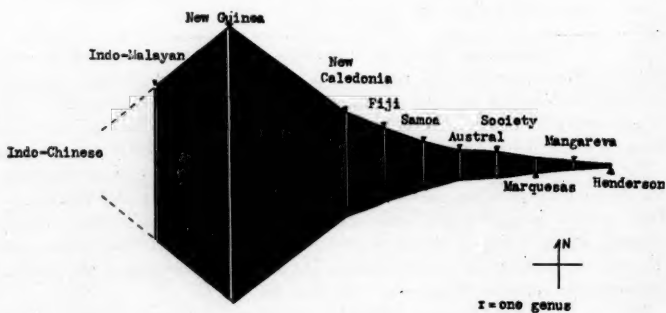
8

SPECIFIC DISTRIBUTION OF MUTILLIDAE

9

GENERIC DISTRIBUTION OF CRYPTOMYXININAE

10



caught in Oligocene pitch. However, there seems no reason for believing that an area with a rich contemporary cockroach fauna existed since the Carboniferous just because cockroaches were dominant among the Carboniferous insect faunas. Certainly we can not assign a flora or fauna to an age older than that indicated by the fossil record. Conifers were present in the early Paleozoic, but flowering plants had their great development in the Cretaceous. Miocene times saw the increase of grasses and culmination of the mammals. It is said that higher birds came in the Eocene, but perching birds are not well represented until the Pleistocene. Perhaps the best fossil histories of animals concern the vertebrates. We are told that the horse began in the Eocene as a small four-toed creature about the size of a small dog, and passed through a number of family and generic changes, and ended in the group of large, single-toed species of *Equus* in Pleistocene and modern times. Although the ancestors of the horse are believed to have originated in America, they did not reach Europe until the Pliocene and found their way to Africa in the Pleistocene. The earliest known ancestors of elephants come into the fossil

FIG. 8. Diagram showing the distribution of the subgenera of mosquitoes from New Guinea to the Marquesas.

FIG. 9. Diagrams showing the specific and subspecific distribution of the Mutillidae (velvet wasps). This is a continental group of heavy-bodied, parasitic wasps whose females are wingless. None have been found on oceanic islands.¹³

FIG. 10. Diagram showing the distribution of the genera of Pacific Cryptorhynchinae (Coleoptera, Curculionidae). This chart is based upon the distributions of several hundred species contained in more than 100 genera. Probably no other subfamily of insects in the Indo-Pacific is so diversified and extensively developed as this one. How like a great funnel fitted with graded filters this chart is. If one turns the figure on its side so that the point is downward, it may be more effective, and one can visualize the barriers between the islands acting as finer and finer sieves or filters that progressively exclude more and more genera as one progresses from west to east. My data on the developments of the genera west of New Guinea are not so complete as I should like to have them. However, the information at hand indicates that the greatest diversification of genera centers around New Guinea and that in the Malay Peninsula and India the number of genera is smaller.

¹³ C. E. Mickel, *Trans. Royal Ent. Soc. London*, 83: 177-312, 1935.

picture at a somewhat later date than the horse. Such data based on careful observation and comparison are lacking in fossil histories of insects. The fossil record of the cockroach is probably the most complete among insects, and it is none too good. Unfortunately, the Mesozoic is mostly *terra incognita* to paleoentomology.

Confronted with such data presented by paleontologists and the conclusions of geologists that the eastern oceanic islands date no farther back than late or middle Tertiary, I am inclined to believe that a considerable amount of the insect speciation within these islands may be called comparatively rapid. I do not believe that the insect faunas of eastern Oceania contain any elements that demand any great age for the developments that have taken place.

It is well to keep in mind that rapid multiplication—especially in the tropics—may be conducive to rapid speciation among insects. The number of offspring arising in a year from a single pair of insects is often surprisingly great. Rapid reproduction of large numbers of short-lived individuals provides more opportunity for mutation. It would be expected that, in general, specific evolution in insects would take a more rapid course than in mammals. Some insects may have 40 or more generations a year. Most of them are short-lived; they live for a year or a season or less. New blood from a new generation is, more often than not, available to each succeeding generation. There is a great mixing of genotypes, and crossing between one pair is unlikely to occur more than once. Most mammals live several to many years and the same blood flows longer. There appear to be no indigenous elements in the eastern oceanic insect faunas that demand as great or greater age for their developments as for such creatures as the horse and elephant, or, for that matter, man, who, it is believed, developed from Miocene primates derived from the Eocene lemuroides of North America.

In the groups I have studied, the continental species

are usually more distinct from their allies than are many of those found among insular species complexes. Muir¹⁷ found the same condition among insular Homoptera, and wrote, "While working on material from the Hawaiian Islands one finds that in many instances 'species' have not the same value as among continental faunas, and one hesitates to give many forms that status, . . ." Maulik¹⁸ expressed the same opinion when studying insular leaf beetles and said, "in insular faunas species tend to become more plastic than in continental faunas." Systematists can usually distinguish species quite readily, but there are natural species complexes existing that defy attempts at facile classification. These complexes are natural, and we may expect them to exist. They are particularly common among oceanic insects and terrestrial mollusca.

Many naturalists cling to the theory of extreme slowness in evolution. Some organisms undoubtedly change slowly, and many have passed through eons of time with little visible mutation. Scorpions have come down from Silurian seas, when trilobites were their contemporaries, unchanged except for minor details that fit them for life on land. Modern genetics and paleontology have shown that such slow change is not universal; it is exceptional. Mutations, even great mutations, occur alike in wild populations and in laboratory or field cultures, though, because they are mostly recessives, they are not often seen in nature. Thus Dobzhansky¹⁹ cites Romashov as finding a population of *Drosophila*, containing a large number of flies homozygous for an aborted wing character, breeding on fruit in a deep ditch. When disturbed, the normal flies flew away and left the flightless flies isolated in the ditch. *Drosophila* studies have shown that mutations may be brought out as homozygous forms in the laboratory by controlled isolation and inbreeding.

¹⁷ *Proc. Haw. Ent. Soc.*, 3 (3): 171, 1916.

¹⁸ *Insects of Samoa*, 4 (3): 191, 1929.

¹⁹ *Biological Reviews*, 14: 355, 1939.

In islands built by successive lava flows and pyroclastic ejecta the formulation of species through isolation may be facilitated by the production of "kipukas"—areas of laterized volcanic soil surrounded and isolated by, but not covered by later lava flows. In these micro-ranges the indigenous flora and fauna remain undisturbed while those in the surrounding areas are destroyed by fresh lava and ash, which can sustain a climax association of plants and animals only after long periods of weathering. Some Hawaiian "kipukas" contain an endemic flora—the survivors of former wider-ranging species now elsewhere extinct.

It seems probable that it is not so much the ages of the islands alone that accounts for the endemic developments of their floras and faunas, but their distinctiveness records prolonged isolation²⁰ to a greater degree and age to a lesser degree. A rule may be formulated thus: The greater the age and isolation of a high eastern oceanic island the greater will be the percentage of endemism and peculiar types of life. The more anomalies in the isolated fauna, the greater is the age. Age alone, without isolation, is not conducive to distinctiveness. The size of a normal high oceanic island is not as important as its age in the development of plants and animals.

Most of the Hawaiian Islands are older and all are more isolated than Tahiti, and the Hawaiian flora and fauna are much more distinctive than are those of Tahiti. Hawaii is younger than Fiji, but Hawaii is more isolated and has more peculiar developments.

No one method seems essential in the formation of oceanic species complexes. The problem is extraordinarily complex. The combination of factors involved in the evolution of land snails with their sedentary habits may not be the same as those influencing flying insects. Romanes²¹ considered that heredity, variation and isola-

²⁰ Isolation from other high oceanic islands as well as continents or continental islands, and not only greater spacial isolation but more constant, more prolonged isolation.

²¹ "Darwin and after Darwin," 1897.

tion formed a tripod on which evolution is based. For more stability of this foundation I would add another leg—that of extinction. These four factors are inseparably linked together, but perhaps the most important to insular speciation is isolation. Isolation should receive much stress in discussions of insular speciation—not only spacial isolation, but also ecological, physiological and morphological.

It might be expected that a genus of flightless insects is more suited to speciation than winged forms. However, the absence of wings does not appear to be more conducive to speciation on oceanic islands than is the presence of wings. *Hyposmocoma*, with about 220 Hawaiian species, is winged: *Proterhinus* in Hawaii, with 175 species, is wingless. Both winged and wingless genera in various orders present great species complexes.

The genitalia of most insects are complex mechanisms. In many groups it is physically impossible for the male of one species to cross with the female of another. It has been said that the key of the male fits only the lock of the female of his own species; although that statement may not be universally true, it holds good in many groups. The genitalia of Microlepidoptera are so individually distinct that, without seeing the entire insect, Mr. August Busck, an international authority on the group, can identify one out of several thousands of species if given the genitalia alone. If differences in genitalia inhibit crossing, then mutations of genital structures should be conducive to speciation. It might even be said that if genitalia are subject to common mutation, this method of isolation might account for insular species complexes, such as those found in *Hyposmocoma*, *Microcryptorhynchus*, and *Proterhinus*.²² Experiments²³ show that female

²² We have too little basic knowledge in this field to go far with our discussions. Professor Dobzhansky, after reading this section, wrote me that "The problem of mutations in genitalia or in other isolating mechanisms interests me greatly. I confess, I am rather lost in the woods in this respect. An 'isolating' mutation, if it is even neutral in all other respects, will be, at least at the start, selected against, since its carrier has less chance to

mammals can be immunized against the sperm of their own species so that the spermatozoa are rendered inactive and quite incapable of fertilization, or, if fertilization does take place, abortion or absorption of the embryo may result. If some mutation occurred that changed the physiological set-up in the genital tract, a cryptic factor of isolation would be at work. Gulick²⁴ has pointed out that if this phenomenon occurred congenitally in the genital tract, it might set up a sterility between human types. The shape and action of the spermatozoa of two allied forms might be so distinct that penetration of the ova of any but one form would not occur.

In a recently published paper, Stebbins²⁵ points out that polyploidy influences not only the morphology but also the physiology and fertility of the plant species. To me, one of the most remarkable discoveries of modern genetical studies is that by means of polyploidy a sterile hybrid can be made to produce a fertile constant species; that not only can some different species be crossed by using polyploid individuals, but that generic crosses can be made by multiplying the number of chromosomes in one to approach the number in another. Stebbins says that "Polyploidy, . . . tends to break down genetic barriers and to permit exchanges of genes between genetic systems that in the diploid condition are completely isolated from each other." "The evidence from the Plant Kingdom, as a whole . . . suggests that polyploidy has been most important in developing large complexes and widespread genera; but that in respect to the major lines of evolution, it has been more important in preserving

produce offspring by crossing to a majority of the species individuals than a 'normal' individual has. Why and how can we visualize the establishment of a mutant which is immune to the sperm of most of its neighbors? One may invoke the help of isolation, but even so the problem is an extraordinarily difficult one, and I see no entirely satisfactory solution."

²³ M. F. Guyer and P. E. Claus, *Physiological Zoology*, 6 (3): 253-288, 1933.

²⁴ A. Gulick, "John Thomas Gulick, Evolutionist and Missionary," 1932.

²⁵ *AM. NAT.*, 74 (750): 54-66, 1940.

relics of old genera and families than in producing new ones." Stebbins calls attention to the fact that polyploids are the ones most apt to expand into new areas and take over new ecological niches. He cites the redwood as a polyploid relic. If there is as much truth as theory in this polyploid research, it may be shown some day that much responsibility for the formation of large insular species complexes among plants can be attributed to polyploidy. Polyploidy does not apply to insects, however.

Controlled crosses that produce organisms which essentially are new species are made by geneticists. In fact, the forms produced are so distinctive that they would probably be described as rare new species if they were collected in the field. If such combinations can be made in the laboratory, doubtless nature herself may now and then produce new forms with like rapidity. I can not conceive of a slow, gradual change bringing forth the flightless Hawaiian Diptera or Neuroptera from their close allies. These aberrant species more probably have had their origins in abrupt mutations of their ancestral stocks. The flightless brown lacewings of Hawaii have arisen, I believe, independently from four different fully winged ancestral species on four different islands and probably at four different times. I have shown²⁶ that among the cryptorhynchine weevils of Rapa there has been a splitting-up of groups of species which, if continued, along with extermination of intermediates, would form various species groups which would be called genera, ultimately to be placed in different tribes. Rapa is an insular laboratory; what has happened and is happening there is obvious. There has been enacted on that small, isolated island a story similar to that played innumerable times on other islands and continents. There appears to be every reason to believe that comparative sudden mutations of forms have occurred in Rapa, and not a slow, gradual change from one type to another. However, I do not wish to imply that I believe all insular

²⁶ *Bishop Mus. Bull.*, 151, pp. 1-75, 1938.

species are the results of large, abrupt mutations rather than series of smaller mutations. Probably there is truth in both theories.

The opinions here expressed regarding rapid speciation are not in agreement with those of some leading students of Mollusca. The fossil record shows that many molluscs are apparently slow in evolving; some species have gone through ages of geological time with little change. Dr. C. Montague Cooke, Jr., has shown me specimens of some Hawaiian genera that have apparently lain fossil for thousands of years, and which do not appear different from present forms. In general, however, it appears to me that most of the Pacific island groups are by no means slow in developing molluscan species complexes. Most oceanic land snails are sedentary animals, and isolation of individuals is easily attained. Different forms are found in areas separated by only a few yards. I am told that isolation of colonies on different trees or in different rock piles is so complete that the individuals of the different colonies take on distinctive facies. The very fact that many endemic land snails set up local colonies at every opportunity is to me indicative of rapid change and speciation. Speciation among oceanic land snails is a field open to genetic investigation and rich with ripe fruits for the student.

V. SUMMARY AND CONCLUSIONS

The high islands of the central Pacific harbor peculiar endemic insect faunas of predominantly small forms normally belonging to few genera containing large numbers of closely allied species, having absolute or almost absolute uni-insular endemism. With the exception of some uncertain elements in Hawaii, these endemic faunas have been derived from western Pacific ancestors. There is nothing in these insect faunas to indicate any great age for their developments and nothing that would demand extensive land area or land bridges in the Pacific. In the south Pacific, the diversity and richness of the fauna of

any high island is inversely proportional to the distance between that island and the Papuan region.

An important problem confronting students of Pacific floras and faunas is the elimination of erroneous data: the broad conclusions based on incomplete collections, meager information or small groups of organisms. There has been too much assumption and too little actual knowledge. In order to assign a given island to its correct position in our scheme, it is essential to take the sum of the floras and faunas and derive the average from them, and to recognize that chance, working over eons of time, is of paramount importance. Abnormal, rather than normal, conditions account for the origins and dispersals of eastern oceanic insular life.

STUDIES ON THE CREEPER FOWL

XIV. THE JAPANESE BANTAM FOWL

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Among the Bantam breeds of fowl Japanese Bantams stand out as typical micromelic dwarfs with proportions of extremities to body closely resembling those of Creeper chickens (Fig. 1). The tibia and fibula of typical Japa-



FIG. 1. A Japanese Bantam cock.

nese Bantams show on dissection an appearance very similar to that observed in Creepers.

It is known among breeders of Japanese Bantams that in the progenies of such birds long-legged, *i.e.*, normally proportioned, individuals appear, and these are excluded from further matings by experienced exhibition fanciers.

In view of these facts it seemed of interest to determine whether or not Japanese Bantams are genetically Creepers; hence, crosses were made between the two breeds.

TABLE 1
INCUBATION DATA FOR VARIOUS MATINGS

Pen and year	Mating	Number of fertile eggs	Mortality during incubation periods			Hatched	Late homozygotes	Late homozygotes per cent. of expected homozygotes
			1-6	7-17	18-22			
20-1937	Creepers ♀♀ × Japanese Bantam ♂	442	actual 77 per cent. 17.4	28 6.3	69 15.6	268 60.6	34 7.7	30.8
9-1939	Creepers F ₁ Inter se × Black Rose Comb Bantam ♂	705	actual 233 per cent. 29.3	31 3.9	181 22.8	350 44.0	10 1.3	5.2
20-1939	Japanese Bantam ♀♀ × Creepers F ₁ ♂ from (Creepers ♀♀ × Black Rose Comb Bantam ♂)	134	actual 28 per cent. 20.9	9 6.7	20 14.9	77 57.5	3 2.2	8.8
20-1938	Creepers F ₁ Inter se from (Creepers ♀♀ × Japanese Bantam ♂)	897	actual 202 per cent. 22.5	114 12.7	234 26.1	347 38.7	59 6.6	26.3

This had to be done by artificial insemination, since the size differences between the two types of fowl preclude successful natural mating. A preliminary note about the results of these crosses has been published (Landauer, 1938); a more detailed account is given in the following report.

A cross of Creeper females by a Japanese Bantam cock (Pen 20, 1937; Table 1) produced 442 fertile eggs. A mortality of 17.4 per cent. occurred during the first few days of incubation and 7.7 per cent. of the embryos showed all the typical features of late Creeper homozygotes (abnormalities of eyes and head, phokomelia). A few additional homozygotes may have died during the middle period of incubation when dead embryos were not examined. The appearance of phokomelic embryos seemed to provide direct proof for the conclusion that the Japanese Bantams carry the Creeper factor or an allele of it, and the figures for embryonic mortality, on the whole, account satisfactorily for the 25 per cent. of lethals expected in a mating in which both parents were heterozygous for the Creeper mutation. The survival rate of homozygotes to late stages is higher than in ordinary Creeper matings. Additional evidence with regard to this peculiarity will be discussed below.

The progeny of this Creeper by Japanese Bantam mating showed the following segregation according to leg length:

	Actual		Per cent.	
	Creeper	Normal	Creeper	Normal
Hatched	214	143	59.9	40.1
Dead embryos	128	51	71.5	28.5
(18-22 days)				
Total	342	194	63.8	36.2

When Creepers are mated inter se the progeny is expected to be composed of two-thirds Creepers and one-third normal chicks. The data given above nearly fulfil this expectation. It was found in our breeding experiments with Creeper fowl that there is generally a small excess of Creepers among the chicks which failed to hatch and a corresponding deficiency of Creepers among the

hatched chicks, indicating a somewhat reduced viability (hatchability) even in the heterozygous condition (*Landauer and Dunn, 1930*). The figures given above demonstrate the presence of the same situation in the cross of Creeper and Japanese Bantam fowl.

We shall next examine data obtained from inter-se matings of short-legged Japanese Bantams. The two matings of this kind for which we have adequate data produced the results shown in Table 2. These data are

TABLE 2
SEGREGATION IN MATINGS OF JAPANESE BANTAMS INTER SE

Mating	Hatched		Dead embryos (18-22 days)		Total	
	Creeper	Normal	Creeper	Normal	Creeper	Normal
33-1937						
actual	163	105	30	22	193	127
per cent.	60.8	39.2	57.7	42.3	60.3	39.7
33-1938						
actual	91	37	74	39	165	76
per cent.	71.1	28.9	65.5	34.5	68.5	31.5
Total						
actual	254	142	104	61	358	203
per cent.	64.1	35.9	63.0	37.0	63.8	36.2

consistent with the expectation for a 2:1 segregation of Creeper and normal offspring, as would occur in inter-se matings of individuals which are heterozygous for the Creeper lethal. It is worthy of note, however, that there was no preferential pre-hatching mortality of Creepers in either one of the two matings.

Classification into "Creeper" and normal offspring, according to leg length, was made at hatching time and confirmed later. No difficulties were encountered in this respect. All short-legged Japanese Bantams used in our crosses proved to be heterozygous for the lethal mutation.

The incubation records for matings of Japanese Bantams are given in Table 3. As compared with similar data for ordinary Creepers, two facts are of interest, *viz.*, the mortality of embryos during the first few days of incubation is consistently below 25 per cent. and the number of homozygous embryos which survived to late stages (and showed phocomelia) is relatively high. In our experiments with ordinary Creeper fowl the incidence of

TABLE 3
INCUBATION DATA FOR MATINGS OF JAPANESE BANTAMS INTER SE

Pen and year	Number of fertile eggs	Mortality during incubation periods				Hatched	Late homozygotes	Late homozygotes in per cent. of expected homozygotes
			1-6	7-17	18-22			
33-1937	206	actual	33	20	64	89	21	
		per cent.	16.0	9.6	31.1	43.2	10.2	40.8
33-1938	357	actual	55	37	133	132	16	
		per cent.	15.4	10.4	37.3	37.0	4.5	17.9
26-1939	65	actual	10	5	19	31	5	
		per cent.	16.4	7.5	26.9	49.3	7.7	30.8
33-1940	87	actual	14	12	31	30	4	
		per cent.	16.1	13.8	35.6	34.5	4.6	18.2
Total	715	actual	112	74	247	282	45	
		per cent.	15.7	10.4	34.5	39.4	6.3	25.2

late homozygous embryos was generally less than 2 per cent. The highest frequency of phokomelic embryos which we ever observed was 6.3 per cent. and occurred in a small mating of Marquesan Creeper fowl; the next highest incidence of late homozygous embryos was 3.7 per cent. in a cross of Scotch and German Creepers. In all our numerous other Creeper crosses we found a much lower average frequency of homozygous embryos which survived the early lethal stage than we did in the two exceptions just mentioned. The matings of Japanese Bantams (Table 3) and the cross of Creeper by Japanese Bantam (Table 1), on the other hand, all gave average survival rates for the homozygous embryos of between 4.5 per cent. and 10.2 per cent., *i.e.*, about 18 to 41 per cent. of all homozygotes lived beyond the early incubation stages and developed into embryos showing phokomelia. The highest incidence of late lethal embryos, recorded for any individual mating, occurred in the progeny of a hen of cross-bred Japanese Bantam stock (♀3780, 1940) bred to a Japanese Bantam male. Among a total of 73 fertile eggs 17 late lethal embryos were found, *i.e.*, 23.3 per cent. of all fertile eggs or 93.4 per cent. of the expected 25 per cent. of homozygotes.

The lowering of the lethal effect of the Creeper mutation in the Japanese Bantam breed seems to apply also to the heterozygous condition, thus accounting for the lack of preferential mortality of heterozygous Creepers previous to hatching (Table 2).

It is known that growth relationships and the proportionality of parts in Bantam breeds differ to a certain extent from those of the larger breeds of fowl (*Lerner*, 1937). For this reason, it seemed of interest to determine whether or not the modified effect of the Creeper mutation in Japanese Bantams is brought about by the general Bantam genotype. Accordingly, Creeper females were bred to a Black Rose Comb Bantam male and the Creeper progeny from this cross (nearly identical with Bantams in body size) was mated inter se (Pen 9, 1938). The results of this experiment are shown in Table 1. We had 795 fertile eggs from this mating: mortality during the first few days of incubation was 29.3 per cent. and only 1.3 per cent. of phokomelic embryos were found. It may be concluded from these data that the size factors and other hereditary agencies, typical of Bantam fowl in general, do not by themselves suffice to influence the chances of homozygous Creeper embryos to survive to late stages.

In another experiment Japanese Bantam females were mated first to a Japanese Bantam cock (Pen 26, 1939; Table 3) and subsequently to a Creeper cock from the cross of Creeper by Black Rose Comb Bantam (Pen 26, 1939; Table 1). In the cross with a Japanese Bantam male 6.3 per cent. of all fertile eggs contained late homozygous Creeper embryos, whereas only 2.2 per cent. of the fertile eggs from the mating with the (Creeper \times Rose Comb Bantam) F_1 cock produced such progeny.

There is evidence from various sources for the conclusion that the higher rate of survival to late stages of homozygous Japanese Bantam embryos, as compared with embryos from ordinary Creeper matings, is caused by genetic modifiers, and that these modifiers are carried by many, but not all, Japanese Bantams. Table 4 gives the frequency of late homozygous embryos for all those Japanese Bantam hens which in any one year produced sixteen or more fertile eggs. It can be seen that, with one exception (Q3601), all hens for which we have records for more than one year either consistently produced late homozygous lethal embryos or consistently did not

TABLE 4

FREQUENCIES OF LATE HOMOZYGOUS LETHAL EMBRYOS IN THE PROGENIES OF INDIVIDUAL JAPANESE BANTAM HENS AS FAR AS THEY PRODUCED SIXTEEN OR MORE FERTILE EGGS DURING ONE LAYING YEAR. ALL BIRDS IN THESE MATINGS WERE KNOWN TO BE HETEROZYGOUS FOR THE LETHAL MUTATION.

Females producing late homozygous lethal embryos			Females which did not produce late homozygous lethal embryos	
Number of hen	Number of fertile eggs	Number of late homozygous embryos	Number of hen	Number of fertile eggs
Pen 33-1937			Pen 33-1937	
3601	17	1	3604	38
3609	31	3	3612	16
3613	27	7	3615	16
3614	36	6		
Pen 33-1938			Pen 33-1938	
3601	29	2	3604	30
3613	26	3	3611	21
3614	45	1	3622	39
3623	25	1		
3625	30	3		
3630	18	3		
Pen 33-1940			Pen 33-1940	
3613	25	4	3622	40
			3601	19
Total				
6 ♀♀	291	31	5 ♀♀	219
Late lethal embryos in per cent. of fertile eggs		10.5		

do so. Among the latter, we had 68 fertile eggs from ♀3604 and 79 fertile eggs from ♀3622 without securing a single late homozygous lethal embryo from either mother; among the former, ♀3613 produced 14 phokomelic embryos out of 78 fertile eggs and for ♀3614 the corresponding figures are 7 phokomelic embryos among 81 fertile eggs.

Attention has already been called to the fact that in the cross of ordinary Creeper females to a Japanese Bantam male (Pen 20, 1937, Table 1) more late homozygous Creeper embryos were found than have ever been observed in any mating of ordinary Creeper fowl. This suggests that the modifying factors are at least partially dominant. When the Creeper progeny of the Creeper by Japanese Bantam mating was intercrossed (Pen 20, 1938; Table 1) the incidence of late homozygous Creeper embryos was again high (6.6 per cent. among 897 fertile eggs, or 26.3 per cent. of all expected homozygous embryos).

An examination of the frequencies of late homozygous lethal embryos in the progenies of individual females of

the Creeper F_1 mating from the Creeper by Japanese Bantam cross (Table 5) shows that all but one or two of

TABLE 5

INCIDENCE OF LATE HOMOZYGOUS LETHAL EMBRYOS IN AN INTER SE MATING OF CREEPER F_1 FROM THE CROSS OF CREEPER $\text{♀} \times$ JAPANESE BANTAM ♂ (PEN 20, 1938): ALL FEMALES WHICH PRODUCED MORE THAN TWENTY-FIVE FERTILE EGGS ARE INCLUDED IN THIS TABULATION.

Number of hen	Number of fertile eggs	Number of late lethal embryos	Late lethal embryos in per cent. of fertile eggs
3651	47	3	6.4
3654	43	3	7.0
3656	57	6	10.5
3657	31	3	9.7
3658	55	3	5.5
3660	49	3	6.1
3663	37	4	10.8
3664	32	3	9.4
3666	44	3	6.8
3668	53	1	1.9
3670	50	2	4.0
3674	55	8	14.5
3675	83	6	7.2

the hens, from which we had more than twenty-five fertile eggs each, gave higher survival rates of the lethal embryos to late stages than would be expected to occur in matings of ordinary Creeper fowl. In none of these individual matings did all the lethal mortality take place at the early stage, though this holds for the majority of individual matings involving the lethal factor of ordinary Creepers.

Such results might be expected in the event of the following alternatives: (1) if the modified lethal effect were due to multiple factors; (2) if it were caused by a gene linked with the Creeper mutation; or (3) if the lethal mutation in the Japanese Bantam stock were an allele of that in ordinary Creepers; in addition, (4) the possibility must be considered that the absence of a clear-cut segregation with regard to the survival time of the F_2 lethal embryos, discussed above, might be caused by a maternal effect (on structure and/or composition of egg constituents).

Some light is shed on the situation by the following experiments. Normal-legged F_1 females from a Creeper by Japanese Bantam cross were mated to an ordinary Creeper cock. From the resulting F_2 generation the Creeper individuals were bred to each other. We had

five such matings, involving thirty-two pullets and five cockerels (Table 6). In none of these matings did more late homozygous lethal embryos appear than might be expected in a mating of ordinary Creepers, and there were no individual females which produced an unusually high

TABLE 6
INCUBATION RECORDS FOR INTER SE MATINGS OF CREEPER F_2 FROM THE CROSS OF
NORMAL F_1 ♀♀ (EX: CREEPER × JAPANESE BANTAM) × CREEPER ♂

Mating	Number of fertile eggs		Mortality during incubation periods			Hatched	Late lethal embryos (actual and in per cent. of fertile eggs)
			1-6	7-17	18-22		
21-1939 with ♂ 4261	214	actual per cent.	66 30.8	15 7.0	49 22.9	84 39.3	3 1.4
21-1939 with ♂ 6235	129	actual per cent.	36 27.9	7 5.4	13 10.1	73 56.6	4 3.1
21-1939 with ♂ 3923	225	actual per cent.	68 30.2	14 6.2	50 22.2	93 41.3	2 0.9
25-1939 with ♂ 4999	120	actual per cent.	28 23.3	11 9.2	19 15.8	62 51.7	0 0
25-1939 with ♂ 3609	456	actual per cent.	132 28.9	24 5.2	66 14.5	234 51.3	8 1.8
Total	1144	actual per cent.	330 28.8	71 6.2	197 17.2	546 47.7	17 1.5

percentage of phokomelic embryos. If the modifying effect, found in Japanese Bantams, were due to multiple factors which are genetically independent of the Creeper mutation, one would have expected at least some of these matings to produce an increased percentage of late lethal embryos. For similar reasons it appears unlikely that the increased average survival rate to late stages of the lethal embryos, in crosses involving Japanese Bantam fowl, is produced by a maternal effect.

It has been pointed out that the less drastic effect of the Creeper mutation, as found in Japanese Bantams, is characteristic for the great majority of, but not all, birds belonging to this breed. Among ordinary Creepers, on the other hand, early death of the lethal embryos is the rule and individual matings which produce appreciable numbers of phokomelic embryos are relatively rare. It seems likely that the prolonged survival of the lethal embryos is produced by modifying agencies which occur with different frequencies in the two breeds of fowl.

All our results are consistent with the hypothesis that the less harmful effect of the Creeper mutation in Japanese Bantam stock is caused by a partially dominant modifier which is fairly closely linked with the Creeper factor. It is possible, however, that in addition to one major modifier additional minor ones exist. Our evidence also does not rule out the possibility that the mutation of Japanese Bantams is an allele to the ordinary Creeper mutation rather than identical with it, but even in this eventuality the existence of at least one modifying gene must be assumed.

SUMMARY

It has been shown that the Japanese Bantam breed of fowl carries the Creeper mutation or an allele of it.

In matings of Japanese Bantams a much larger percentage of homozygous embryos generally survives to late developmental stages than in matings of ordinary Creeper fowl.

In contradistinction to the situation in matings of ordinary Creepers, no preferential embryo mortality of heterozygous Creepers occurs in Japanese Bantam stock.

The results of our breeding experiments suggest that the modified effect of the Creeper mutation in Japanese Bantams is caused by an incompletely dominant modifier linked with the Creeper factor.

ACKNOWLEDGEMENT

The work which has been reported here was started on the basis of suggestions and information received from Rektor Walter Rüst, Nowawes, Germany. I wish to record my indebtedness for his interest.

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REVIEWS AND COMMENTS

EDITED BY CARL L. HUBBS

IN this section reviews and notices are given of current publications on general biology and of specialized works which have an important bearing in this general field. Emphasis is given to books and major articles which fall within the special scope of *THE AMERICAN NATURALIST*, in that they deal with the factors of organic evolution.

REVIEWS AND COMMENTS are meant to include also such general discussions, reports, news items and announcements as may be of wide interest to students of evolution. Except as otherwise indicated, all items are prepared by the Section Editor, Dr. Carl L. Hubbs, University of Michigan, Ann Arbor, Michigan. All opinions are those of the reviewer.

Adaptive Coloration in Animals. By HUGH B. COTT. New York: Oxford University Press, 1941: i-xxxii, 1-508, col. frontisp., pls. 1-48, figs. 1-84. \$8.50.

IN this Neodarwinian epic Dr. Cott stamps himself as a true disciple of the master evolutionist. Indeed, he rivals Darwin in the thorough, objective and penetrating analysis of a major biological problem. An immense body of facts and interpretation, much of it original, has been judiciously considered and brought to bear on the question of the biological significance of coloration. The adaptive value of colors is very convincingly indicated.

Probably concerned over the limited amount of critical experimental data on the survival significance of concealing colors, the author selects for special consideration the clear-cut results of the American naturalists, Sumner and Isely, both of whom have demonstrated that relative protection is afforded animals whose colors match those of their backgrounds. Cott seems convinced, however, that the adaptive value of colors is also shown by the general lore of natural history. The harmony between observation and theory, the almost universal impressions of field naturalists, the cumulative confirmation of the idea—all indicate to him that the colors of animals accord vital protection.

This convincing treatise is one element in the resurgence to Darwinian views, which is leaving some biologists, particularly in America, stranded above the tide. The author's general philosophy of adaptation is

sound. It is clear to him that each animal which has survived must have become marvelously adapted; that each kind is adapted in many peculiar ways. We may carry the argument on to the logical conclusion: should an animal become so highly adapted as to be immune to attack, it would in time lose the special protective features which had been evolved, just as surely as long-term residents in darkness lose their pigment and eyes. Conversely, the less the immunity, the greater the selection, for only under severe predatory pressure can highly adaptive features be secured and maintained. Perfection of adaptation, in protective coloration, for example, is a tribute to the ability that the predators have evolved in finding and destroying the protected prey. The prey have thereby been forced to further adaptation. Consequently, the destruction of highly adapted animals must ordinarily be great—though not quite so great, were these animals not so well adjusted for survival. In this view it seems utterly illogical to challenge the theory of adaptation, on the finding that supposedly protected animals are eaten, even when consumed in large numbers.

In section after section, rivaling one another in fascination, this master of art and of natural history unfolds the biological significance of adaptive coloration in animals. He shows how animals in general are cryptically colored; how they may vary with locality, with changing habitat, with age and with the season, so that protection is given at various places and times. He demonstrates beyond any hesitant doubt the value of countershading or "obliterative shading," which is exhibited by virtually all animals in which it could be expected to operate and is reversed in some which swim or hang upside-down. Ruptive markings are shown to be oblitative also, on both optical and biological grounds. The attainment of maximum disruptive contrast, through the light margining of dark streaks and the dark edging of light bands, is proposed as a new generalization (the reviewer arrived at the same idea, in studying the coloration of the reef fishes

of the West Coast, from 1916 to 1926). Particularly impressive is the author's treatment of "coincident disruptive coloration," in which a ruptive mark crosses structural boundaries, so as to obliterate visually such ordinarily conspicuous parts as the eye and the limbs. Concealment of an animal's ordinarily telltale shadow is also stressed.

Having thus portrayed the methods by which concealment is attained in nature, Cott discussed the function of concealing coloration, in defense and in offense. He then answers with devastating logic the objections which have been raised to the theory of concealing coloration, and stresses the effectiveness of this principle.

In similar thoroughness the author treats, as "advertisement," the methods by which conspicuousness has been attained by aposematic animals; how they appear and behave, how warning displays are made, and how the aposematic colors of some animals are made use of by commensals. The protective attributes of aposematic animals, the relation between warning colors and distasteful attributes, the effectiveness of these combined protective characteristics, are then treated. It is shown that vertebrate enemies have the capacity to learn by experience, and the selective feeding propensities, that are called for on the theory that some colors have a warning significance and are protective.

The large volume ends with the phase of adaptive coloration which has proved most fascinating to adherents, but which has seemed most unsound to the critics—namely, mimicry and related phenomena. Cott uses a general classification of "disguise" to include the special concealing resemblances of animals to leaves, bark, lichens, lianas, excrement, algae, etc. He then reviews the peculiar behavior patterns which so dramatically and indubitably enhance some of these resemblances. Conspicuous localized color patterns, of both deflective and directive significance, are then considered. Misplaced eyes and head-like features on the tail ends of animals

are included. The most highly evolved of the adaptive resemblances—alluring coloration and mimicry—are reserved for final consideration.

This book is the work of an artist, and it is a work of art. Every biologist with an interest in any phase of natural history or evolution should keep it at hand.

College Entomology. By E. O. ESSIG. New York: Macmillan Co., 1942: i-vii, 1-900, frontisp., figs. 1-308. \$5.00.

COMPREHENSIVE, authoritative, detailed, useful, are the attributives which come to mind in examining this new compendium of the insects. The treatment is essentially systematic. The first two chapters briefly deal with metamorphosis and anatomy, particularly as these subjects bear on the identification of the 33 recognized orders and almost innumerable families of insects. Economically important and typical representatives of the various groups are briefly discussed, and many of them are figured.

Immunity Against Animal Parasites. By JAMES T. CULBERTSON. New York: Columbia University Press, 1941: 1-274, 4 pls., 3 figs. \$3.50.

DR. CULBERTSON'S thoroughgoing and workmanlike presentation of the recent contributions to our knowledge of immunity against animal parasites is not a book for beginners. In no sense a "popular" book, it avoids arguments as well as personal concepts and theories which are not supported by experimental data.

The basic style of the book is simple, forthright and clear, but the totality of references cited and the minuteness of detail will prove overwhelming to any but the advanced student. In short, it is a source book of considerable value written with skill. Unfortunately, the author has made no attempt to evaluate the contributions of various workers and one finds questionable speculative data given the same consideration as well-controlled meticulous work. One notes no citations in the introduction to the pioneer studies on the flagellates by Novy and

colleagues, although international recognition followed their appearance.

The material is arranged under three headings: Natural Resistance and Acquired Immunity; Immunity in Specific Diseases; and Applied Immunology. In part one, an attempt is made to list and explain the various factors which are generally recognized and associated with active immunity. Particular attention is given to age. In the second section, starting with the amoebiasis and ending with the arthropods, immunity to specific diseases is considered in a uniform and possibly monotonous manner. The final chapters are devoted to such practical problems as the stimulation of active immunity and procedures of aid in the diagnosis of animal infections.

The book will meet the needs of the research student, who will be appreciative of the immense amount of time that has gone into the assembling of the references.

MALCOLM H. SOULE

SYMPOSIUM ON CRITERIA FOR VERTEBRATE SUBSPECIES,
SPECIES AND GENERA

THE criteria for the recognition of the minor taxonomic categories of vertebrates were discussed at a symposium held at the American Museum of Natural History on April 3, under the joint auspices of the American Society of Ichthyologists and Herpetologists and the American Society of Mammalogists. The fishes were treated by Carl L. Hubbs; the lower systematic categories in herpetology, by E. R. Dunn; subspecies, species and genera in ornithology, by Ernst Mayr; the same, in mammals, by E. Raymond Hall; criteria for genera, species and subspecies in zoology and paleontology, by George Gaylord Simpson; and criteria for species and their subdivisions from the point of view of genetics, by W. Frank Blair.

AMERICAN SOCIETY OF NATURALISTS

At its annual meetings held in connection with the American Association for the Advancement of Science

at Dallas, the American Society of Naturalists elected R. E. Cleland, Indiana University, as *President*, Dr. L. C. Dunn, Columbia University, as *Vice-President* for the next year, and Dr. M. R. Irwin, University of Wisconsin, as *Treasurer* for the next three years. Dr. A. C. Kinsey, Indiana University, continues as *Secretary* for a period of three years.

At the same meetings, the following persons were elected, on account of outstanding achievement in biological research, as members of the society: B. G. Anderson, Western Reserve; P. B. Armstrong, Syracuse; L. G. Barth, Columbia; F. A. Beach, American Museum of Natural History; C. M. Breder, Jr., New York Aquarium and American Museum; J. W. Buchanan, Northwestern; W. Burrows, Chicago; D. P. Costello, North Carolina; J. N. Couch, North Carolina; P. S. Galtsoff, U. S. Bureau of Fisheries; V. Hamburger, Washington; A. Hollaender, U. S. Public Health Service; C. G. Huff, Chicago; L. H. Hyman, American Museum of Natural History; G. W. Kidder, Brown; L. H. Leonian, West Virginia; H. S. Liddell, Cornell; N. E. McIndoo, U. S. Bureau of Entomology; C. P. Oliver, Minnesota; R. Rugh, New York; F. O. Schmitt, Washington; G. L. Stebbins, Jr., California; I. R. Taylor, Brown; H. B. Tukey, Cornell; B. W. Wells, North Carolina State College; E. T. Wherry, Pennsylvania; D. M. Whitaker, Stanford; T. G. Yuncker, DePauw.

CONSERVATION OF SCHOLARLY JOURNALS

THE American Library Association created this last year the Committee on Aid to Libraries in War Areas, headed by John R. Russell, the librarian of the University of Rochester. The committee is faced with numerous serious problems and hopes that American scholars and scientists will be of considerable aid in the solution of one of these problems.

One of the most difficult tasks in library reconstruction after the first World War was that of completing foreign institutional sets of American scholarly, scientific and

technical periodicals. The attempt to avoid a duplication of that situation is now the concern of the committee.

Many sets of journals will be broken by the financial inability of the institutions to renew subscriptions. As far as possible they will be completed from a stock of periodicals being purchased by the committee. Many more will have been broken through mail difficulties and loss of shipments, while still other sets will have disappeared in the destruction of libraries. The size of the eventual demand is impossible to estimate, but requests received by the committee already give evidence that it will be enormous.

With an imminent paper shortage attempts are being made to collect old periodicals for pulp. Fearing this possible reduction in the already limited supply of scholarly and scientific journals, the committee hopes to enlist the cooperation of subscribers to this journal in preventing the sacrifice of this type of material to the pulp demand. It is scarcely necessary to mention the appreciation of foreign institutions and scholars for this activity.

Questions concerning the project or concerning the value of particular periodicals to the project should be directed to Wayne M. Hartwell, Executive Assistant to the Committee on Aid to Libraries in War Areas, Rush Rhees Library, University of Rochester, Rochester, New York.

SHORTER ARTICLES AND DISCUSSION

CONTRIBUTORS to the AMERICAN NATURALIST will please note that material for the "Shorter Articles and Discussion" has not been received in desired balance with the longer manuscripts. It is hoped that this section may be expanded somewhat. Notes and discussions that are appropriate to the field of the journal can probably be handled with less delay than will be entailed in putting the main articles through the press.

SHORTER ARTICLES AND DISCUSSION

ENGRAILED, A PUPAL LETHAL AT HIGH TEMPERATURE IN *DROSOPHILA HYDEI*

INTRODUCTION

THE occurrence of true parallel mutations in two different species of *Drosophila* has been proved by the direct test of allelism in hybrids (Sturtevant, 1929). However, where the species can not be crossed strong evidence may still be found in similarity of phenotype, particularly when this is of a polymorphic nature, and in the association of the mutants in question with a particular chromosome arm considered homologous in the two species. Thus a large series of sex-linked mutants in many species of *Drosophila* seem almost certainly to be true parallels.

Among autosomal mutants the strongest case for parallelism may be made when the mutant in question is not represented by mimics (similar mutations at different loci) within the species, when the expression is polymorphic or pleiotropic, and finally when accompanied by other apparent parallels in the same chromosome arm in two or more species (Sturtevant and Novitski, 1941).

ENGRAILED IN *DROSOPHILA MELANOGASTER*

On November 7, 1926, K. Evang found the mutant "engrailed" in a *Drosophila melanogaster* stock. This is the only recorded occurrence of a mutant of this type in the species. Eker (1929) has listed the following phenotypic manifestations: a median notch in the hind border of the scutellum, varying to completely bifurcated scutellum; wings larger and broader, bluntly rounded at the end and thin-textured; wing veins altered, with sections missing and extra pieces of vein added. Brasted (1941) has described a secondary sex-comb opposite the primary comb on the fore-tarsi of males. She also reported unpublished observations of Mrs. Viola Curry on a variable rotated genitalia character in engrailed males.

Moriwaki (1937) has given a brief description of an autosomal dominant in *Drosophila ananassae* which he names "engrailed." He lists only the variable character, nicked scutellum, and states that the mutant is a recessive lethal. Without further evidence

it does not seem valid to consider this a parallel. Neither is the mutant "bipectinate" in *Drosophila simulans* (Sturtevant, 1929) mentioned by Brasted as similar to engrailed in melanogaster to be considered as a true parallel.

ENGRAILED IN *DROSOPHILA HYDEI*

Spencer (1939) has listed an "engrailed" mutant in *Drosophila hydei*, recovered in 1937 from inbreeding offspring of a pair of wild flies from a citrus dump near Azusa, California. Subsequent study of temperature effects on the expression of this mutant are of interest particularly in connection with the extensive work of Brasted (1941) on the melanogaster mutant.

The evidence that the engrailed mutants in melanogaster and hydei are true parallels follows. *Drosophila hydei* engrailed is in the same linkage group with a vestigial-like mutant and cinnabar (the latter shown to be a parallel of cinnabar in melanogaster by interspecific eye-disc transplantation test made by Dr. G. W. Beadle). In *Drosophila melanogaster* engrailed, vestigial and cinnabar occur in the right limb of the second chromosome.

Of the engrailed characters listed above for melanogaster, notched and double scutellum, ragged venation with additions and deletions of vein sections, rounded, broad and thin-textured wings are observable in *Drosophila hydei* engrailed under certain environmental conditions. Rotated genitalia has not been found. The secondary sex-comb is not present. This is, however, not surprising, as there is no primary sex-comb in this species. The probable parallel nature of the hydei "engrailed" was first called to the author's attention by Dr. A. H. Sturtevant.

In view of the range of expression among alleles at a single locus in many mutant types within a species it is not surprising to find some differences in degree of expression between apparently parallel mutants in different species. It also seems likely that the same allele would manifest itself somewhat differently both in quality and quantity expression against the dissimilar genetic backgrounds of two diverse species such as melanogaster (subgenus *Sophophora*) and hydei (subgenus *Drosophila*). For this reason the study of parallel mutations may contribute valuable data on questions of developmental genetics.

In *Drosophila hydei* under certain environmental conditions the following additional characters may be observed in engrailed: doubling or bifurcation of the third antennal joint, accompanied

by reduction or total loss of the arista; shortened tarsi with distal joints missing and plate-like outgrowths on tarsi even varying to presence of branched tarsi; partially split thorax with incomplete doubling of all dorsal thoracic structures; as many as eight scutellar bristles when scutellum is split; eyes bulging or reduced and roughened; wings blistered.

TEMPERATURE EFFECTS

Great variability was noted in different stocks of engrailed kept at room temperature and in incubators. Sometimes all flies were normal except for slightly thickened wing veins, particularly the posterior cross-vein. At other times most of the flies emerging were extreme variants. The following experiment was run to determine possible temperature effects.

From engrailed parents all of which showed slight phenotypic expression young larvae were separated into three lots and placed in large shell vials on a cleansing tissue-yeast suspension medium, a culture method which we have found optimum for rearing

TABLE I
POLYMORPHIC EXPRESSION OF ENGRAILED IN 192 ADULTS FROM
LARVAE REARED AT 24° C.

Thick veins	Short scut.	Short tarsi	No. scut. bristles	Blister wing	Arista absent	Bifurcate antenna	Split thorax	Split scut.	Male	Fem.
+	+	+	2	+	+	+	+	+	1	0
+	+	+	5	+	+	+	+	+	1	0
+	+	+	6	+	+	+	+	+	3	2
+	+	+	4	+	+	-	+	+	0	1
+	+	+	6	+	+	+	+	+	1	0
+	+	+	2	+	+	-	+	+	1	0
+	+	+	5	+	+	-	+	+	0	1
+	+	+	4	+	+	+	+	+	0	1
+	+	+	6	+	+	+	+	-	0	1
+	+	+	6	+	+	-	+	-	1	1
+	+	+	3	+	+	+	-	-	0	1
+	+	+	4	+	+	+	-	-	5	5
+	+	+	5	+	+	+	-	-	5	9
+	+	+	6	+	+	+	-	-	19	38
+	+	+	7	+	+	+	-	-	7	5
+	+	+	8	+	+	+	-	-	3	5
+	+	+	4	+	+	-	-	-	2	3
+	+	+	5	+	+	-	-	-	5	5
+	+	+	6	+	+	-	-	-	25	13
+	+	+	6	-	+	+	-	-	0	4
+	+	+	7	+	+	-	-	-	2	1
+	+	+	8	+	+	-	-	-	3	2
+	+	+	6	+	-	-	-	-	5	0
+	+	+	6	-	+	-	-	-	1	0
+	+	+	7	+	-	-	-	-	3	0
+	+	+	8	+	-	-	-	-	1	0
Totals									94	98

+ denotes presence of character; - denotes normal.

hydei. One lot was reared at 20° C, a second at 24° C and the third at 30° C.

Of the 207 flies classified from the 20° C vials all showed slight thickening of the wing veins, particularly the posterior cross-vein. One also had blistered wings. Otherwise the flies were normal. The larvae reared at 30° C went through development to apparently normal pupation, but never emerged as adults. Wild-type controls at this temperature were quite normal in eclosion and in the appearance of the imagos. Thus engrailed proved an effective lethal, acting in the pupal stage at 30° C.

At 24° C the flies all showed moderate to extreme expression of the engrailed phenotype. Table I gives the complex of characters shown in each of the 192 flies reared at this temperature. With our method of culture flies reared at 24° C are as large as those at 20° C and wild-type at 30° C are equally large. By providing a soft substrate, cleansing tissue and a very rich food, yeast suspension, the error of secondary effects due to small size at high temperature may be eliminated.

Qualitative observations on cultures at temperatures somewhat above 24° C indicated no falling off of phenotypic expression at higher temperatures and dissection of a few pupae reared at 30° C showed extreme misshapen individuals. Brasted found that the secondary sex-comb character was most strongly expressed at 14° C and that most flies at 29° C did not have a secondary sex-comb. Scutellum notching was strongest at 25° C with expression falling off at lower and higher temperatures. Fig. 1 is an

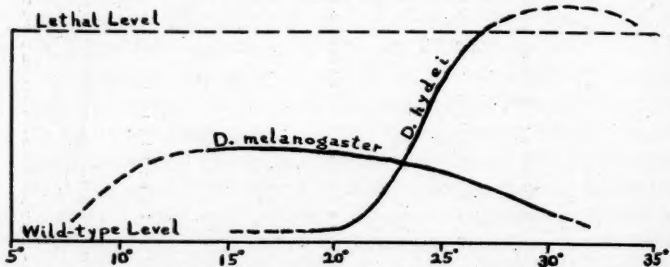


FIG. 1. Approximate curves of phenotypic expression of the mutant "engrailed" in two species of *Drosophila*.

attempt to represent graphically the total phenotypic action of temperature on engrailed in the two species.

The melanogaster allele with which we are concerned may be a mild one. Or, it may be that the time relationships in growth

processes in this species are such as to allow for only weak phenotypic expression of engrailed alleles. On the contrary, the hydei allele may be a strong one or the time relationships in this species may be such as to bring about extreme expression of a mutation at the engrailed locus. If it were possible to rear *melanogaster* at temperatures much lower than 14° C the expression of engrailed might be brought as close to wild-type as in the case of hydei at 20° C. Conversely, if it were not that the engrailed expression in hydei runs into the lethal area at between 24° C and 30° C it might be possible to demonstrate a drop in the phenotypic expression of some characters at high temperatures. These possibilities are suggested in the figure by dotted line interpolations of the curves.

In general it may be said of the hydei mutant that the expression tends to follow the pattern of structure duplication followed by loss. Thus organ-forming stuff may be so distributed as to produce doubling (bifurcated antennae, tarsal outgrowths, extra venation); but if spread too thin the morphogenic threshold is not reached and veins drop out, tarsal joints disappear and arista fail to form.

SUMMARY

An engrailed mutant in *Drosophila hydei* is almost normal when reared at 20° C; shows more extreme expression at 24° C than the engrailed mutant of *Drosophila melanogaster* in its most extreme manifestation; and is an effective pupal lethal when larvae are reared at 30° C. The difference of expression in the two species may be accounted for in terms of their diverse genetic systems.

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BATH HABITS OF CANARIES

THESE canaries have lived for many years uncaged in the laboratory. Their bath pan is twelve inches by twenty-two, the running water kept at three quarters of an inch. If deeper, certain birds will not risk to jump in. A spout serves as a shower. Some go regularly under the shower, some get under accidentally, and some carefully avoid it.

A baby may bathe as early as the first day out of the nest, when approximately twenty-one days old. One big-bodied male jumped into the bath on his first day, but just stood, seemed not to know what to do, so was lifted out, whereupon he started right back, teeter-tottered on the rim of the pan, fell in, had a vigorous bath that struck you afresh for the immediate perfection of the inherited steps. But another had not jumped in by its seventh month, would loiter by the edge, occasionally splash itself by slapping its bill on the water or even place itself in the spray of some bird which was bathing.

The following is the order of the usual bath. The canary hops to the rim, from there looks alertly around the laboratory and does not begin if there is any even remote danger. Then it drinks several swallows. Then it wets its face, which it is apt to wash first. Then it wades in, dips, rises quickly from that first dip. Then it dips again and this time definitely submerges chest and abdomen, rises and in rising flaps its wings, which throws the drops over its back. This last succession of steps is repeated three to fifteen times, with increasing energy and at shorter intervals, the drops higher and wider. Suddenly the bird jumps out of the bath and walks away, but may as suddenly walk back and jump in again. I have seen a bird leave and return nine times, throughout examining itself over and over apparently to see how sufficient its work. The drying is by running, by flying with violent changes of direction, by shaking, by picking single drops off the feathers, by drawing the long feathers through the bill and so stripping off the water, and by wiping, especially the face, and, finally, if there is sun, by spreading in it. There is a lame bird that wipes on a towel. The towel was there by chance first, then was laid out deliberately, and nowadays if it is forgotten the bird variously asks for it.

The bath is a frequent scene of fights. Such fights occur commonly for one of two reasons. One, a bird is advancing toward the bath, but is still dry, does not want to be splashed till it

splashes itself, and accordingly protests. Two, a bird is in the bath, wings heavy with water, does not want another to come near enough to block its escape if that were to become necessary, and accordingly protests.

Imitation counts much. A bird happens to alight near the water, so gets in, and within five minutes half the population will have had a bath. Or a bird takes off from the food table to the bath—or from the bath to the food table—and whereas one minute many were concentratedly eating, the next minute many will be concentratedly bathing and drinking. Furthermore, likes occupy the pan at the same time, the all-white, the crested, the half-breeds—at least, this is so frequent as to make it striking to any eye. A change in the neighborhood of the bath—if the outlet gets stuffed and the water rises—may first excite caution, but then, oddly, may excite a brisk general bathing.

A chilly day reduces the number of bathers, but with established cold weather the baths are about as frequent in winter as in summer. On such a chilly day there will be periods when you see knots of birds huddle near the pan, plainly “think” bath, but end by only one or two taking a drink. A sharp but sunny day, on the contrary, is sure to be a powerful and successful stimulus.

The individuality of the single canary is revealed at the bath as at every focus of canary life. Early morning is the time of choice for the bath, yet certain ones never bathe till afternoon. None bathes so late as not to leave ample time for drying, yet there is one which quite systematically takes a small bath late at night. The old bathe generally less, as you see by the dusty shades of their feathers, nevertheless the three oldest, all over seventeen years, have bathed regularly through their lives. One male bathes frequently, five times on a single hot afternoon, and on such an afternoon may be seen to let the shower stream monotonously down his back, and afterward not employ the customary great muscular effort required to dry, but he lets the day dry him. A curious fact is that after the scrubwoman has cleaned the laboratory there is an invariable rush for the bath, especially those few days of the year when the place is fumigated, and what you then always think is: With everything else clean the birds want to be clean too. One of them takes ice baths. These began when a refrigerator pan was left standing with several melting ice cubes. Since that time on any warm noon you

may see this bird bathing with the icebergs all around it. And from bathing in ice water it took to eating ice cream. I have seen its wings so wet from the ice bath that it could not fly, but must ascend the food table by hopping up a small ladder, where it ate the ice cream. An occasional canary bathes in sand. All bathe in sun. Two with sore feet bathed just their feet, soaked them for a considerable time. One with a sore anus soaked that by definitely squatting in the water, would stay quite stirless and also for a considerable time. There is one male sings in his bath. Another I have seen jump in, then pipe back and forth with his female, and get so interested in this duet that his bath could not start. One eats in his bath.

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MORPHOLOGICAL COLOR CHANGE IN THE CRAYFISH

INTRODUCTION

THE great variety of color in Crustacea and the ability of these animals to adapt themselves to different backgrounds has interested many investigators. Briefly, Crustacea fall into three categories in respect to pigmentation: (1) Most cave Crustacea are colorless or nearly so (Packard, 1888); (2) Crustacea from mid-depths of the sea are brilliantly colored, generally scarlet, and there is usually lack of any pattern, (Alcock, 1902; Murray and Hjort, 1912); (3) the body color of other Crustacea usually harmonizes with the surroundings, especially in the smaller and more delicate forms.

Much has been reported in the literature concerning physiological color changes in Crustacea, *i.e.*, concentration and dispersion of the pigment within the chromatophores in response to sinus gland extracts and light intensity. However, there has been very little work done on morphological color changes. Keeble and Gamble (1904) suggested that in *Hippolyte varians* there is an increase in the rate of formation of new chromatophores in the regions of the animal where the chromatophores are expanded, and that in this way the adult color pattern is fitted to the environment. However, no experiments were carried out to test this hypothesis. Morphological color changes in fishes have been investigated by many biologists, who have shown that the chro-

matophores of one color increase in number when the background causes the continuous dispersion of their pigment and decrease when the background causes the pigment's concentration. For a review of this see Sumner (1940). On the other hand, with the exception of one set of experiments by Brown (1934), there has been no investigation of morphological color changes in crustaceans. Brown (1934) made extracts of the pigments of *Palaeomonetes* and compared the concentration of the pigment in the extracts with standard solutions by means of a colorimeter. He found that on a white background, in light, red and blue pigments were very rapidly destroyed, while the yellow disappeared slowly. On a black background, in light, red and blue pigments were formed. Shrimps kept in darkness at first lost, through destruction, their red and blue pigments, but after about two weeks there was a reversal of this process. Red and blue pigments were most rapidly formed and destroyed; yellow and white pigments were much more slowly changed. Brown (1934) believes it possible that pigment formation and destruction in adaptation to colored backgrounds may be controlled by the same humoral agents that are responsible for the migration of the pigment within the chromatophores.

The object of this experiment was to determine whether continued dispersion of chromatophore pigment results in an abnormal increase in the number of these chromatophores, and conversely whether continued concentration leads to a decrease in the number of the chromatophores kept in that condition.

MATERIALS AND METHODS

Young crayfish (*Cambarus clarkii* Girard) were used in this experiment. These were obtained by isolating an adult female with eggs in an aquarium. At the beginning of the experiment counts were made of the number of red and white chromatophores in the telson of twenty-five crayfish from the stock aquaria (brown pebble background) using a dissecting microscope. These will be referred to as "normals." For this purpose a crayfish was immobilized by placing it on an ice cube, and the uropods were spread apart with a camel's hair brush. Red chromatophores are visible in either reflected or transmitted light; white chromatophores are distinguishable only in reflected light. Counts were usually made of chromatophores in the concentrated state for when they were dispersed in was sometimes impossible

to determine where one began and another left off. To facilitate counting, the telson was divided into four parts, and counts were made of each part separately. The length of the carapace of each animal was measured, using a filar micrometer.

Twenty-five crayfish were placed in each of two five-gallon aquaria which had been painted black on the sides and bottom, two ordinary five-gallon aquaria and two white enamel pans. The ordinary aquaria were placed in a darkroom during the experiment. The animals were fed once a week with enchytrae, and the water was changed at least once a week, usually twice a week. Counts were made after the animals had been in the aquaria from 52 to 110 days.

RESULTS

Table 1 sums up the data on all animals.

TABLE 1

	Ave. no. days on back- ground	Ave. no. of red chromato- phores	Ave. no. of white chromato- phores	Ave. per cent. of white chromato- phores in relation to total. \pm standard error	Ave. length of cara- pace (mm)
Normals	432.6	61.8	12.9 ± 0.57	4.8
Black background ..	91	523.4	51.3	8.9 ± 0.68	5.6
Dark room	86	1031.6	186.6	15.3 ± 1.02	7.9
White background ..	88	909.6	240.4	20.9 ± 2.34	8.0

To be significant the difference between any two means (average per cent. of white chromatophores) must be at least twice as great as the standard error of the difference (σ_d). σ_d is equal to the square root of the sum of the squares of the two standard errors. Table 2 gives the differences between the means and σ_d for each of the six possible pairs. Differences between the means are to the left of the diagonal line; σ_d is to the right of the diagonal line.

In each case the difference between the means is significant; thus the effect of background on the ratio of red to white chromatophores is significant. Some of the increase in the absolute numbers of red and white chromatophores in animals kept on black and white backgrounds and in the dark room was due to normal growth, uninfluenced by background. This growth is shown by the carapace measurements. On a black background there is an increase in the relative number of red chromatophores.

TABLE 2

	N	W	D	B
N		2.41	1.17	0.89
W	7.98		2.74	2.44
D	2.40	5.58		1.23
B	4.00	11.98	6.40	

N, normals.
W, white background.
D, dark room.
B, black background.

On a white background there is an increase in the relative number of white chromatophores. The situation in the dark room is intermediate, although significantly different from the normal animals.

Four of the animals which had been on a white background for three months were placed in individual black finger bowls after counts had been made. After more than two months they were still in the white-adapted condition; *i.e.*, the white pigment was maximally dispersed and the red pigment completely concentrated or only slightly dispersed. This may be explained by the hypothesis that the red pigment in the erythrophores was so little in quantity that it was unable to fill more than the center of the cell. Conversely, there was so much white pigment in the white chromatophores that it filled the entire cell.

DISCUSSION

Crustaceans thus may adapt themselves to their surroundings by both physiological and morphological color changes. Physiological color change is a relatively rapid process, while marked morphological color change requires, in the crayfish, weeks or months. Morphological color change consists of modification, in response to background, of the relative number of the different kinds of chromatophores and of the amount of pigment in each chromatophore. It provides protective coloration to an extent that would be impossible if physiological color change were the only means of providing background adaptation, and limits the extent to which physiological color changes can be effective.

Because of variations in size, it was impossible to determine whether the effect of background was to cause an abnormal increase in the number of chromatophores in which the pigment was expanded, or to cause a subnormal increase or even decrease in the number of chromatophores in which the pigment was concentrated, or whether both took place simultaneously.

SUMMARY

1. Counts were made of the number of red and white chromatophores in the telson of three lots of young crayfish (*Cambarus clarkii*) after they had been on a white background, a black background and in the dark respectively for 52 to 110 days. These were compared with counts made on animals from the stock aquaria before the experiment was initiated.

2. The average number of white chromatophores in relation to the total number of chromatophores decreased on the black background; and increased on the white background. In the dark there was a slight increase over the normals. The situation in regard to the average number of red chromatophores in relation to the total number of chromatophores was the converse of the white.

3. Some of the animals were unable to become black-adapted after they had been on a white background during the course of the experiment. It is suggested that this is due to a large increase in the amount of white pigment in the individual white chromatophores, and a concomitant decrease in the amount of red pigment in the individual red chromatophores.

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